Published in the United States of America

2012 • VOLUME 5 • NUMBER 3

AMPHIBIAN & REPTILE CONSERVATION

amphibian-reptile-conservation.org

ISSN: 1083-446X

eISSN: 1525-9153

Editor

Craig Hassapakis
Berkeley, California, USA

Associate Editors

Raul E. Diaz
University of Kansas, USA

Howard O. Clark, Jr.
Garcia and Associates, USA

Erik R. Wild
University of Wisconsin-Stevens Point, USA

Assistant Editors

Alison R. Davis
University of California, Berkeley, USA

Daniel D. Fogell
Southeastern Community College, USA

Editorial Review Board

David C. Blackburn
California Academy of Sciences, USA

C. Kenneth Dodd, Jr.
University of Florida, USA

Harvey B. Lillywhite
University of Florida, USA

Peter V. Lindeman
Edinboro University of Pennsylvania, USA

Jaime E. Péfaur
Universidad de Los Andes, VENEZUELA

Jodi J. L. Rowley
Australian Museum, AUSTRALIA

Bill Branch
Port Elizabeth Museum, SOUTH AFRICA

Lee A. Fitzgerald
Texas A&M University, USA

Julian C. Lee
Taos, New Mexico, USA

Henry R. Mushinsky
University of South Florida, USA

Rohan Pethiyagoda
Australian Museum, AUSTRALIA

Peter Uetz
Virginia Commonwealth University, USA

Jelka Crnobrnja-Isailović
IBISS University of Belgrade, SERBIA

Adel A. Ibrahim
Ha'il University, SAUDIA ARABIA

Rafaqat Masroor
Pakistan Museum of Natural History, PAKISTAN

Elnaz Najafimajd
Ege University, TURKEY

Nasrullah Rastegar-Pouyani
Razi University, IRAN

Larry David Wilson
Instituto Regional de Biodiversidad, USA

Advisory Board

Allison C. Alberts
Zoological Society of San Diego, USA

Michael B. Eisen
Public Library of Science, USA

Russell A. Mittermeier
Conservation International, USA

Aaron M. Bauer
Villanova University, USA

James Hanken
Harvard University, USA

Robert W. Murphy
Royal Ontario Museum, CANADA

Walter R. Erdelen
UNESCO, FRANCE

Roy W. McDiarmid
USGS Patuxent Wildlife Research Center, USA

Eric R. Pianka
University of Texas, Austin, USA

Antonio W. Salas
Environment and Sustainable Development, PERU

Dawn S. Wilson
AMNH Southwestern Research Station, USA

Honorary Members

Carl C. Gans
(1923 – 2009)

Joseph T. Collins
(1939 – 2012)

Cover:

Neurergus kaiseri. In a pioneering program, Sedgwick County Zoo, Kansas, USA, is breeding for sale the Critically Endangered Loristan Newt (*N. kaiseri*) to support field work and conservation in Iran and to increase stocks with private breeders. *Photo Nate Nelson.*

Amphibian & Reptile Conservation—Worldwide Community-Supported Herpetological Conservation (ISSN: 1083-446X; eISSN: 1525-9153) is published by Craig Hassapakis/*Amphibian & Reptile Conservation* as full issues at least twice yearly (semi-annually or more often depending on needs) and papers are immediately released as they are finished on our website; http://amphibian-reptile-conservation.org; email: arc.publisher@gmail.com

Amphibian & Reptile Conservation is published as an open access journal. Please visit the official journal website at: http://amphibian-reptile-conservation.org

Instructions to Authors: *Amphibian & Reptile Conservation* accepts manuscripts on the biology of amphibians and reptiles, with emphasis on conservation, sustainable management, and biodiversity. Topics in these areas can include: taxonomy and phylogeny, species inventories, distribution, conservation, species profiles, ecology, natural history, sustainable management, conservation breeding, citizen science, social networking, and any other topic that lends to the conservation of amphibians and reptiles worldwide. Prior consultation with editors is suggested and important if you have any questions and/or concerns about submissions. Further details on the submission of a manuscript can best be obtained by consulting a current published paper from the journal and/or by accessing Instructions for Authors at the *Amphibian and Reptile Conservation* website: http://amphibian-reptile-conservation.org/submissions.html

© Craig Hassapakis/Amphibian & Reptile Conservation

Copyright: © 2011 Browne et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Amphibian & Reptile Conservation 5(3):1-14.

Zoo-based amphibian research and conservation breeding programs

[1]ROBERT K. BROWNE, [1,4]KATJA WOLFRAM, [2]GERARDO GARCÍA, [3]MIKHAIL F. BAGATUROV, AND [1,5]ZJEF J. J. M. PEREBOOM

[1]*Centre for Research and Conservation, Royal Zoological Society of Antwerp, BELGIUM* [2]*Durrell Wildlife Conservation Trust, Jersey, Channel Islands, UNITED KINGDOM* [3]*Department of Insectarium and Amphibians, The Leningrad Zoo, St. Petersburg, RUSSIAN FEDERATION*

***Abstract.*—The rapid loss of amphibian species has encouraged zoos to support amphibian research in concert with conservation breeding programs (CBPs). We explore "Zoo-based amphibian research and conservation breeding programs" through conducting a literature review and a survey of research publication with public and subscription search engines. Amphibians are ideal candidates for zoo-based amphibian research and CBPs because of their generally small size, high fecundity, ease of husbandry, and amenability to the use of reproduction technologies. Zoo-based amphibian research and CBPs can include both *in situ* and *ex situ* components that offer excellent opportunities for display and education, in range capacity building and community development, and the support of biodiversity conservation in general. Zoo-based amphibian research and CBPs can also benefit zoos through developing networks and collaborations with other research institutions, and with government, business, and private sectors. Internet searches showed that zoo based research of nutrition, husbandry, reproduction, gene banking, and visitor impact offer special opportunities to contribute to amphibian conservation. Many zoos have already implemented amphibian research and CBPs that address key issues in both *ex situ* and *in situ* conservation; however, to reach its greatest potential these programs must be managed by scientific professionals within a supportive administrative framework. We exemplify zoo-based amphibian research and CBPs through the experiences of zoos of the European Association of Zoos and Aquariums (EAZA), the Russian Federation, and the United States.**

Key words. Zoo research, amphibian, conservation breeding programs, Internet searches, Internet surveys

Citation: Browne RK, Wolfram K, García G, Bagaturov MF, Pereboom JJM. 2011. Zoo-based amphibian research and conservation breeding programs. *Amphibian & Reptile Conservation* 5(3):1-14(e28).

Introduction

Official reports estimate more than nearly 158 amphibian species have gone extinct since their description (AmphibiaWeb 2011) and that 30% of the 6726 species of amphibians listed by the IUCN Amphibian Red List (IUCN 2010) are threatened, including 484 Critically Endangered and 754 Endangered species. Over the coming decades threats to amphibians are expected to increase with a corresponding increase in the number of amphibians requiring dedicated management programs (McCallum 2007; Sodhi et al. 2008).

To reduce the rate of biodiversity extinction in general the World Zoo and Aquarium Conservation Strategy (WAZA 2005) committed the world's zoos to include conservation breeding programs (CBPs) supported by research as a key component in their conservation strategies (Baker 2007; Hutchins and Thompson 2008). CBPs prevent species extinction through maintaining genetically representative populations and providing animals for supplementation, rehabitation, or translocation projects (Baker 2009; Shishova et al. 2010; Browne et al. 2011). In 2007 specific support for amphibian CBPs was also provided by the Species Survival Commission of the International Union for the Conservation of Nature (IUCN/SSC) who recommended that CBPs should be implemented where necessary for all critically endangered amphibians (Gascon et al. 2007). To efficiently address the prevention of species loss in 2009 the European Association of Zoos and Aquariums (EAZA) recommended combining CBPs with scientific research, education, and outreach (EAZA 2009).

Correspondence. *Email:* [1]*robert.browne@gmail.com (corresponding author);* [2]*gerardo.garcia@durrell.org;* [3]*bbigmojo@mail.ru;* [4]*Katja.Wolfram@kmda.org;* [5]*zjef.Pereboom@kmda.org*

Figure 1. Research in zoos, such as this study on tadpole growth and development at Antwerp Zoo, can make substantial contributions to conservation breeding programs. *Image by Robert Browne.*

The number of amphibian species that require CBPs is challenging. However, the World Association of Zoos and Aquariums (WAZA) represent 241 zoos in 48 countries, and globally there are more than 1000 zoos and aquariums in zoo and aquarium associations (WAZA 2009). This number is greater than the total number of Critically Endangered amphibians, some of which do not immediately need CBPs and may be perpetuated through *in situ* initiatives. Therefore, the support of amphibian CBPs by zoos' in concert with other institutions should be able to assure a minimal risk of amphibian extinctions.

To achieve the highest benefit to cost ratio the structure of CBPs preferentially should integrate both international and regional capacities (Reid et al. 2008; Ziegler 2010). CBPs in a species' biogeographical or biopolitical range are generally more economical and sustainable than those out of range, and they also provide the advantages of local scientific expertise, capacity building, and community engagement (e.g., Ziegler and Nguyen 2008; Nguyen et al. 2009). Maintaining rescue populations within regions also reduces the chance of pathogen dissemination (Pessier and Mendelson 2010) or the release of invasive species (NBII 2011). Regional universities, government departments, and NGOs can all provide centers for expertise and facilities combined with academic research.

Amphibian CBPs offer zoos, with limited capacity, an attractive alternative to those for large mammals and birds, or with zoos, in general, an opportunity for diversification or extension of their conservation programs. The primary goals of CBPs initially include the building of a genetically representative captive population, and then maintaining health, reliable reproduction, and the perpetuation of genetic variation. However, problems with satisfying these criteria for larger vertebrates (Araki et al. 2007) make the management of zoo-based CBPs for these species expensive and difficult (Lees and Wilcken 2009). Baker (2007) showed that since 2000 the success of CBPs for large, thermoregulating vertebrates has declined due to numerous challenges including insufficient founders, poor health and reproduction, and loss of genetic variation (Hutchins and Conway 1995; Baker 2007). In contrast, amphibians are mostly small, adequate numbers of founders may be sampled and held, are amenable to husbandry, and their reproduction and genetic variation can be managed especially when supported by research (Browne and Figiel 2010; Browne et al. 2011).

Therefore, zoo-based amphibian CBPs can include direct maintenance of genetically competent populations, as well as their use for education, display, and research. They can also extend to other institutions and private keepers and breeders within the international community (Zippel et al. 2010), while offering support to local communities, preserving habitat, supplying surplus amphibians for the pet market, and reducing wild harvesting (Furrer and Corredor 2008; Zippel et al. 2010). Zoo-based amphibian CBPs can sell surplus amphibians to generate funds directly for conservation, gain valuable publicity, and widen the range of threatened species available to private caregivers.

Zoos are housing an increasing number of exhibits supporting amphibian conservation (Zippel 2009; Amphibian Ark 2010). Amphibians are easily kept in attractive exhibits where their role within ecosystems and the reasons for their decline can be presented. Through public education that demonstrates zoos' role in amphibian conservation and research, zoos can function as ambassadors for contemporary best practice in *ex situ* biodiversity conservation (Reid et al. 2008; Ziegler et al. 2011).

Ex situ research for amphibians can vary over a wide range of disciplines including nutrition and husbandry, display and education, population genetics, and reproduction technologies. *In situ* research includes amphibian biodiversity assessment, ecology, habitat preserva-

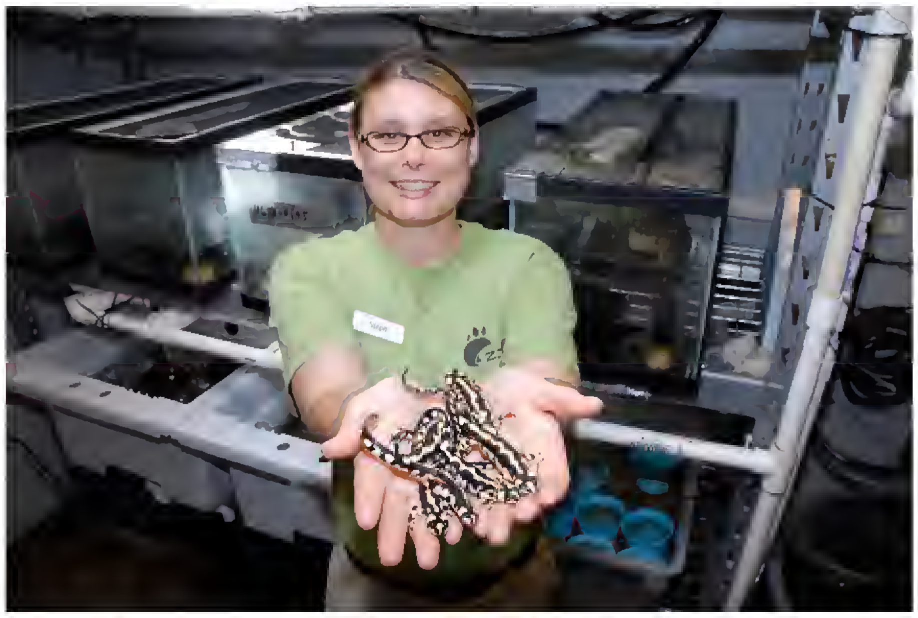


Figure 2. *Neurergus kaiseri.* In a pioneering program, Sedgwick County Zoo, Kansas, USA, is breeding for sale the critically endangered Loristan newt (*Neurergus kaiseri*) to support field work and conservation in Iran and to increase stocks with private breeders. *Image by Nate Nelson.*

tion, and identifying threats and their mitigation (Browne et al. 2009). Therefore, amphibian research in zoos can support both *in situ* and *ex situ* conservation of amphibians, contribute to fundamental science, and can develop valuable scientific and conservation collaborations (Furrer and Corredor 2008; Browne et al. 2009).

In situ aspects of amphibian CBPs offer zoos attractive opportunities to integrate their amphibian conservation strategies with those for general biodiversity. These include the establishment of regional facilities, habitat preservation, and community education that provide a focus for biodiversity conservation and ecosystem sustainability (Lawson et al. 2008). Amphibians with aquatic life stages are particularly susceptible to extinction where threats include water borne diseases (Lips et al. 2003), water pollution (Rohr 2008), and introduction of invasive species (M. Bagaturov and K. Mil`to, pers. comm.).

Table 1. The hits for each term, for a scientific field, as a percentage of all hits (years covered, 1900 to 2009). Searches engines; 1) *Google Scholar*, 2) *PubMed*, 3) *Scopus*, and 4) *ISI Web of Knowledge*.

The percentage of "term" hits of total "scientific field" hits from 1900 to 2009					
Search engine	1	2	3	4	Mean
Scientific field					
Behavior	34	4	19	66	31
Behaviour	9	1	14	21	11
Medicine	21	27	2	7	14
Disease	24	9	8	34	19
Husbandry	7	1	1	1	3
Aquaculture	1	1	1	1	1

Table 2. The hits for each scientific field as a percentage of all hits (for scientific fields: years covered, 1900 to 2009). Searches engines; 1) *Google Scholar*, 2) *PubMed*, 3) *Scopus*, and 4) *ISI Web of Knowledge*.

The percentage of subject hits of total hits from 1900 to 2009					
Search engine	1	2	3	4	Mean
Scientific field					
Behavior/behaviour	23	6	30	47	27
Physiology	6	70	18	11	26
Medicine/disease	25	3	9	16	13
Reproduction	24	1	8	10	12
Genetics	9	17	11	5	11
Diet	8	1	4	6	5
Population genetics	1	1	8	3	3
Husbandry/aquaculture	4	1	2	1	2
Nutrition	1	1	1	1	1

Consequently, many *in situ* components of amphibian CBPs correspond with the conservation needs of threatened freshwater fish, reptiles, birds, mammals, plants, fungi, microorganisms, and invertebrates, including high risk groups like mussels, crayfish, and aquatic plants (Davic and Welsh 2004). In some cases, due to their aquatic and terrestrial life stages and specialized microhabitats, amphibians may also be important bioindicators through complex ecological interactions (Rohr 2008).

We explore "Zoo-based amphibian research and conservation breeding programs" through a literature review, a survey of research effort through public and subscription Internet search engines, and provide examples of successful programs through the experiences of zoos of the European Association of Zoos and Aquariums (EAZA), the Russian Federation, and the United States.

Methods

A survey of research effort in scientific fields relevant to amphibian CBPs was conducted through two publicly accessible databases on the Internet (*Google Scholar* and *PubMed*), and two subscription Internet search engines (*Scopus* and *ISI Web of Knowledge*, volume 4.7). Searches were conducted over the years covered in the databases between 1900 to 2009. Search dates and data were collected on 27 December 2009 (*Google Scholar*, *Scopus*, and *ISI Web of Knowledge*) and 28 December 2009 (*PubMed*).

Search strings for amphibians were based on the following main descriptors: "amphibian [search subject]," "frog [search subject]," "salamander [search subject]," "toad [search subject]." Search strings were chosen for each search engine with a combination of the above descriptors that returned the maximum number of credible hits.

Using the above descriptors, the search subjects of alternative "terms," used to describe "scientific fields," were compared between the numbers of hits from the four search engines (Table 1). For "scientific fields" (alternative terms pooled) we also compared the percentage of hits of each of the total hits from 1900 to 2009 (Table 2).

Results

General: The total number of hits returned for all scientific fields were: *Google Scholar* (1,670), *PubMed* (10,741), *Scopus* (14,528), and *ISI Web of Knowledge* (6,245). *PubMed* indexed the *Medline* database of citations, abstracts, and full-text articles with a total number of indexed citations of more than 19 million. *Scopus* indexed more than 18,000 journals (including 16,500 peer-reviewed), 350 book series, and 3.6 million conference papers. *ISI Web of Knowledge* indexed more than 23,000 journals, 110,000 conference proceedings, and 9,000 websites. *Google Scholar* indexed an undetermined number of full-text articles from most peer-reviewed online journals, as well as citations, websites, and books from the main publishers in Europe and America.

Searches of alternate "terms" for "scientific fields:" Table 1 shows wide and inconsistent differences between search engines in the percentage of hits between alternate "terms" for scientific fields.

Searches of "scientific fields:" Table 2 shows the wide range, in the percentage of hits between search engines, for each term, for each scientific field, between search engines. The percentage of total hits, averaged from all search engines for each term, ranged from 1 to 27%. More than 50% of the average hits were from behavior/behaviour (27%) and physiology (26%), while medicine/disease, reproduction, and genetics comprised about 12% each. Only a small percentage of hits (11%) included diet/nutrition (6%), population genetics (3%), and husbandry/aquaculture (2%).

Discussion

Our Internet search engine survey of amphibian publications showed that search engines varied widely in the number of hits dependent on the terms used to describe the scientific field, and in hits for each scientific field. Therefore, when conducting search engine surveys, alternative subject terms for each scientific field should be compared through an appropriate range of search engines to produce meaningful results (Jansen and Spink 2006; UNEP-WCMC 2009).

There have been relatively few publications on amphibians, compared to other vertebrates, except fish in *Zoo Biology*, where Anderson et al. (2008) showed that from 1982 to 2006 publications mainly concerned mammals (75%), then birds (11%), reptiles (4%), amphibians (3%), fish (2%), and invertebrates (2%).

Anderson et al. (2008) also showed that overall, with vertebrates, some subjects critical to CBPs were poorly represented in zoo research. Publications over all taxa focused on behavior (27%), reproduction (21%), husbandry/animal management (11%), diet and nutrition (8%), veterinary medicine (7%), genetics (6%), anatomy/physiology (6%), and housing enrichment (4%; Anderson et al. 2008). Our Internet search engine survey showed a similar percentage of publication subjects for amphibians as in Anderson et al. (2008) for behavior/behavior and genetics, a higher percentage for medicine/disease, and lower percentages for reproduction, diet, husbandry/aquaculture and nutrition. Our survey also showed that in some fields important to amphibian CBPs, there were relatively few publications concerning medicine/disease, reproduction, and genetics, and even fewer publications on diet/nutrition, population genetics, and husbandry. Therefore, within the needs of CBPs, reproduction, diet, husbandry/aquaculture, nutrition, and genetics offer research subjects of particular value for zoos.

An Internet questionnaire survey of amphibian research efforts in zoos (Browne et al. 2010a) included responses from 89 institutions globally, with 47% of responses from AZA and 10% from each from EAZA, ALPZA, and ZAA/ARAZPA. This survey showed a recent change in emphasis in amphibian research efforts in zoos as a result of zoos' recognition of the value of amphibian CBPs. Research included 23% of institutions supporting wide-ranging research of phylogenetics/taxonomy and 30% supporting research of supplementation, rehabitation, or translocation. *Ex situ* research mainly focused on reproduction (54%), population management and conservation education (40%), diet/nutrition (30%), and disease management (22%). *In situ* research was highest for species conservation assessment (46%) and disease (35%), while 13% investigated each of land/water use, climate change, or introduced species, and 5% of environmental contamination or overharvesting.

Research effort increased over the period from 2008 to 2010, with ~80% of institutions having dedicated research staff and ~50% having space for research or access to museum or university facilities (Browne et al. 2010a). However, only ~35% had dedicated laboratory space or direct research funding, with the majority of funded institutions having less than US$5,000 in research funding. Nevertheless, there was a predicted increased proportion of overall funding in the bracket from US$5,000-50,000 from 2011 to 2013.

The need expressed in the survey for laboratory facilities could be partly satisfied by greater outreach and collaboration with academic institutions. Opportunities for increased scientific collaborations, networking, and provision of projects were also presented as research needs. Sixty percent of respondents had produced popular publications promoting amphibian conservation. There was considerable focus on peer-reviewed publications, with 30% of respondents having published, and 70% currently conducting scientific research for peer-review.

Anderson et al. (2008) showed that there was little direct collaboration between zoos and other institutions on research publications, with only 9% of articles coauthored between zoos and universities. The recent development of zoo research reliant upon professional staff may account for the greater emphasis on collaborative scientific publications. An aspect of zoo-based CBPs and research not investigated by Anderson et al. (2008) or (Browne et al. 2010a) was the embracing of authorship from regions of high amphibian biodiversity. Previous limitations in the breadth of authorship of articles (Newman 2001) are being addressed globally through the Internet, which offers expanding potential for both networking and communication (Olsen et al. 2008).

Six major challenges need to be overcome to achieve successful CBPs: 1) maintaining good husbandry techniques, 2) controlling reproduction, 3) maintaining genetic variation, 4) success in rehabitation, supplementation, or translocation, 5) providing oversight by professional scientific personnel, and 6) the fostering of career development through exchanges, meetings, and training of keepers and amphibian managers. These goals all appear achievable within zoo-based amphibian CBPs with the support of research.

Hutchins and Thompson (2008) found with rehabitation programs, mainly for mammals, that only 12% had established self-sustaining populations. In contrast, amphibian rehabitations were much more successful, where Griffiths and Pavajeau (2008) showed a success rate of 52% between 1991 and 2006. Similarly, Germano and Bishop (2009) found increased success of amphibian rehabitations between 1991 and 2009 in comparison to those before 1991 (Dodd and Siegel 1991). Although these achievements are impressive, Hutchins and Thompson (2008) suggested that further improvements could be made in CBPs through increased long-term research commitments.

In 1986, Soulé et al. published the need for CBPs for thousands of threatened mammal, bird, and reptile species. Due to low founder numbers, large body size restricting the numbers in captive populations, low fecundity, poor health, and difficulties in arranging suitable pairings, few of the established CBPs for mammals, birds, and reptiles are maintaining genetic variation (Baker 2007). Lowered genetic variation results in poor health and reproduction, which reduces the viability of the captive population and the production of competent individuals for release (Baker 2007; Akari et al. 2007; Allentoft and O'Brien 2010).

The small size of amphibians and recent advances in genetics, husbandry, and reproduction technologies, offer zoos the opportunity to develop CBPs with healthy and reproductive amphibians populations, the perpetuation of their genetic variation, and the ultimate goal of providing competent individuals for rehabitation, supplementation, or translocation (Browne and Zippel, 2007a; Burggren and Warburton 2007; Browne and Figiel 2011). The increasing use of gene banking, and particularly the use of cryopreserved sperm, enable the cost efficient and reliable perpetuation of amphibians' genetic variation. Additional cost benefits of gene banking are reduced numbers of individuals required for CBPs (Shishova et. al 2010; Browne and Figiel 2011, Mansour et al. 2011). Zoos are now in an excellent position to facilitate or directly develop reproduction technologies for amphibians (Browne and Figiel 2011; Browne et al. 2010; Shishova et al. 2010). Some zoos and supporting institutions can also now develop gene banks for threatened amphibians that store a range of samples including sperm, cells, and tissues (Browne and Figiel 2011).

However, although fertilization was first achieved with cryopreserved amphibian sperm in 1996 (Kaurova et al. 1996), sperm banks are only now being established that represent the natural genetic variation of any amphibian species. For example, the North American giant salamander (*Cryptobranchus allegianensis*), most commonly called the hellbender (CNAH 2011), is suffering from very low or negligible recruitment over much of their range and only older adults remain. In response, Nashville Zoo at Grassmere, USA, has recently pioneered the sampling of semen over the range of *C. allegianensis* and developed techniques for its sperm cryopreservation and gene banking (National Geographic 2010; Michigan State University 2010). Zoos have played a significant role in the use of hormones to induce reproduction in both male and female amphibians (Browne et al. 2006a, b), and these technologies now promise the reliable reproduction of many species (Trudeau et al. 2010).

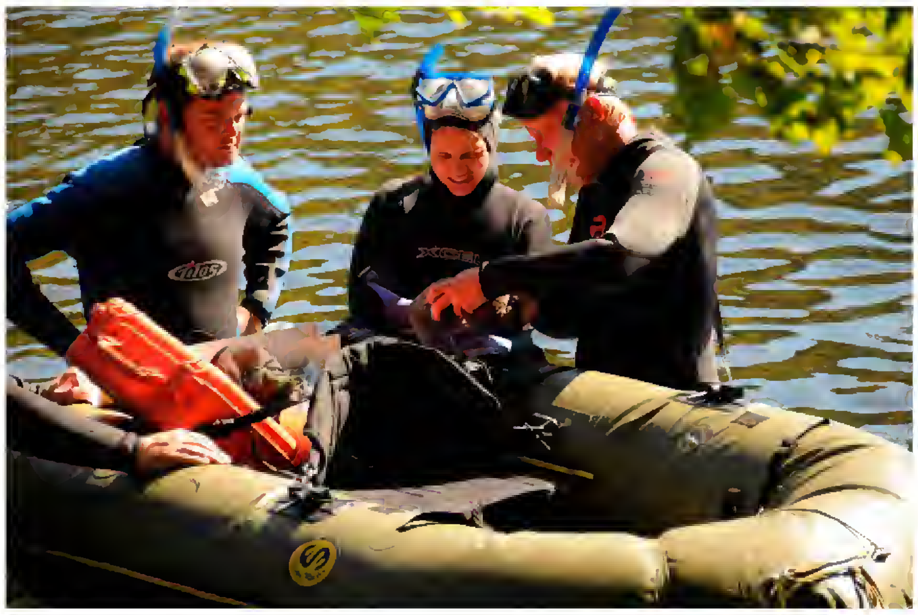


Figure 3. Hellbender sperm sampling. A team led by Dale McGinnity, Nashville Zoo at Grassmere, Tennessee, USA, is creating the first genetically representative gene bank for any amphibian put forth using the hellbender (*C. alleganiensis*). *Image by Sally Nofs.*

Diet and nutrition have a major effect on amphibian health, lifespan, and reproductive output (Li et al. 2009). Historically, research of amphibian diet and nutrition has mainly tested the benefit of dusting feeder insects with vitamin/mineral powder. However, the natural diet of amphibians includes insects with a wide variety of micronutrients. Recent research in zoos has included reviews of Vitamin D_3 deficiency (Antwis and Browne 2009), nutritional metabolic bone disease (King et al. 2010), and the supplementation of feeder insects to avoid vitamin and other micronutrient deficiencies (Li et al. 2009).

To reach their greatest potential, amphibian CBPs should extend to areas where amphibian biodiversity faces the greatest threats (Lötters 2008; Bradshaw et al. 2009). These areas are generally in developing countries of tropical regions where there is high growth in human population (United Nations 2004) and corresponding loss of native vegetation and wetlands (Wright and Muller-Landau 2006a, b), including much of Africa (Lötters 2008).

Specific threats to amphibians that could be incorporated into zoo-based *in situ* research include the loss and fragmentation of wetlands and forests (Bradshaw et al. 2009), emerging diseases (Dazak et al. 1999; Pessier 2008; Skerratt et al. 2007), pollutants and climate variability (McDonald and Sayre 2008; Foden et al. 2008), and unregulated harvest (Mohneke and Ródel 2009). In general, essential *in situ* research components of amphibian CBPs include surveys of range and distribution, pathogen assessment, DNA sampling and population genetics, microhabitat assessment, and autecology (Browne et al. 2009). Relict montain rainforests in tropical regions often provide the only remaining natural habitat for much biodiversity, and these forests are often subject to ongoing vegetation clearance (Lötters 2008; Bradshaw et al. 2009). Zoo research integrated with direct financial support, of the conservation of these relict habitats, could be particularly cost effective.

Many of these conservation initiatives are incorporated into Cologne Zoo's amphibian CBPs within a framework of long-term amphibian biodiversity research and nature conservation (Ziegler 2007; 2010). An Amphibian Breeding Station was established and founded by the Vietnamese and Russian Academies of Sciences at the Institute of Ecology and Biological Resources (IEBR) in Hanoi, Vietnam. Research supported by Cologne Zoo at the breeding station has focused on the ecology, reproduction, and larval identification, development of data-deficient and threatened amphibians, and the commercial breeding of selected species to both decrease over harvesting and provide financial support to help the station become self-supporting. Fourteen out of 21 species have successfully reproduced.

Cologne Zoo and their Vietnamese partners, including the Vietnam National University, Hanoi and IEBR, since 1999 have also conducted long-term biodiversity research at a UNESCO World Heritage Site, Phong Nha-Ke Bang National Park, Vietnam. This project works in concert with forest protection, ranger support, and wildlife rescue. In the past decade, thirteen new amphibian and reptilian species have been described from a small area of 86,000 ha and more than 40 new amphibian species have been described since 1980 (Ziegler et al. 2006, 2010; Ziegler and Vu 2009). Cologne Zoo also supports a CBP for amphibians at their aquarium in Cologne where 16 species have been reproduced in the past decade (Ziegler et al. 2011).

Many other zoos in EAZA have supported programs to develop regional capacity for amphibian conservation, where Durrell Wildlife Conservation Trust, UK, leads a major program for the conservation of the Montserrat mountain chicken frog (*Leptodactylus fallax*; Martin 2007; Garcia et al. 2007). A consortium of zoos and institutions in Europe, Canada, and the USA are building both *ex situ* and *in situ* capacity and research for the critically endangered Lake Oku clawed frog (*Xenopus longipes*; Browne and Pereboom 2009). A similar CBP is established for the critically endangered Kurdistan newt (*Neurergus microspilotus*) and Loristan newt (*N. kaiseri*) between European and USA institutions with Razi University, Iran (Browne et al. 2009).

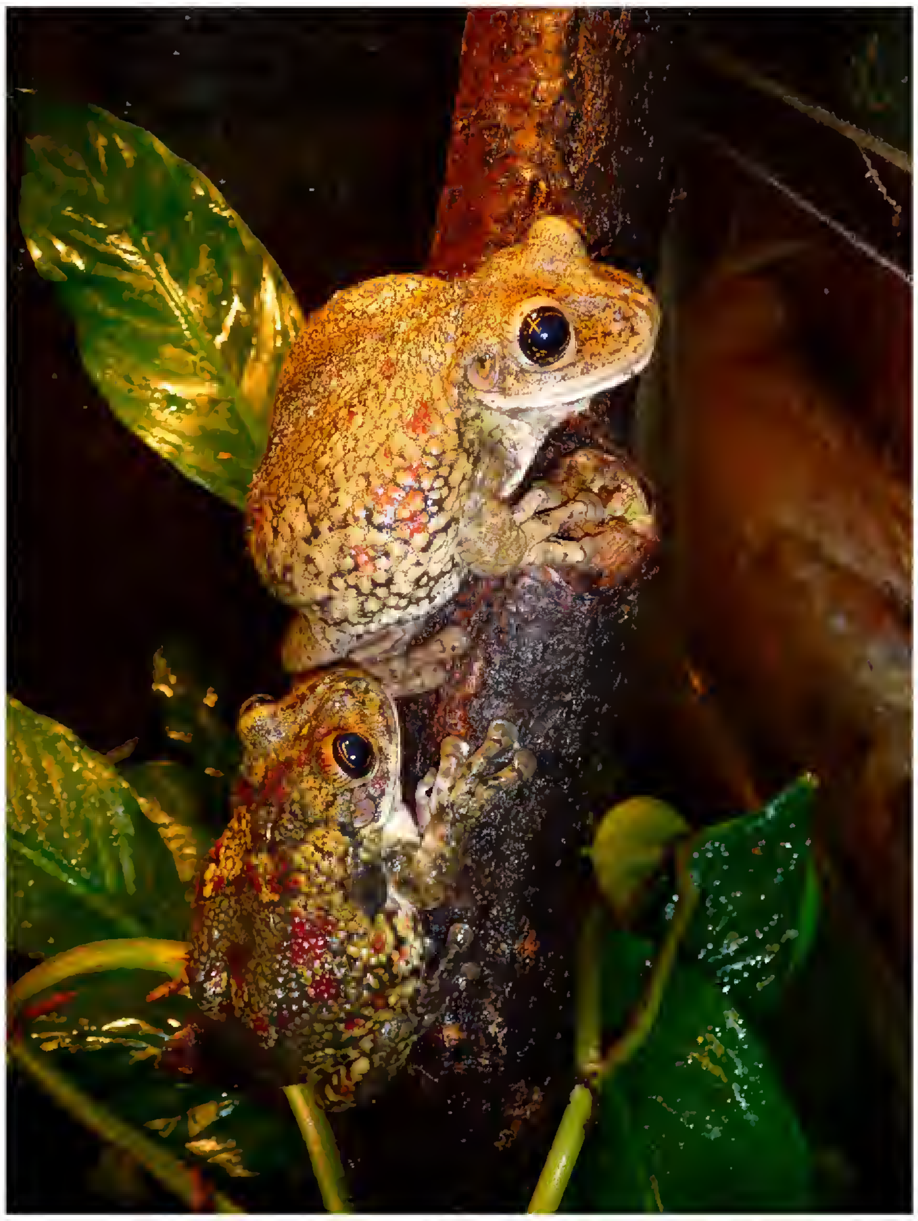


Figure 4. *Trachycephalus nigromaculatus.* The black-spotted casque-headed treefrog (*Trachycephalus nigromaculatus*) is an excellent display species because it is large (10 cm), spectacular, and sits in the open. These frogs are very popular pets in the Russian Federation. *Image by Mikhail Bagaturov.*

Durrell Wildlife Conservation Trust, UK, has head-started Agile frogs (*Rana dalmatina*) in a successful program for their recovery. These skills were then transferred to an *ex situ* and *in situ* program for the Iberian frog (*Rana iberica*) and the Midwife toads (*Alytes obstetricans* and *A. cisternasii*; G. Garcia, pers. comm.). Perth Zoo, Australia, has established a CBP and rehabitation for the White-bellied frog that involves both *ex situ* and *in situ* components (*Geocrinia alba*; Read and Scarparolo 2010). These are only a few examples of the many similar programs being developed globally.

The recently established (2009) Department of Invertebrates and Amphibians in Leningrad Zoo (St. Petersburg, Russia) has developed an amphibian collection of over 80 species. Their *ex situ* programs focus on the reproduction of Asiatic amphibians and has succeeded in reproducing and raising to adulthood over 10 amphibian species, including such rare and threatened species as *Paramesotriton laoensis*, *Rhacophorus feae*, *R. orlovi*, *R. annamensis*, *Theloderma* spp., American species of Dendrobatidae, and several amphibian species of former USSR territories (e.g., *Bombina variegata*; Bagaturov 2011a, b). This work is supported through collaboration

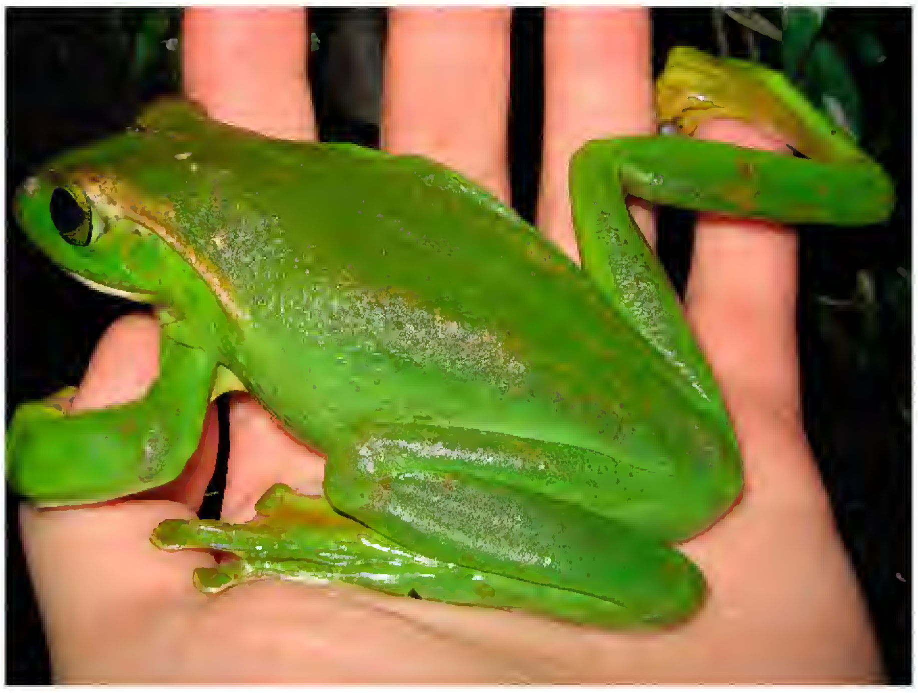


Figure 5. Fea's tree frog (*Rhacophorus feae*) from SE Asia, possibly the largest species of tree frog in the world. Found in high montane forests and recently captive bred for the first time at Leningrad Zoo. *Image by Mikhail F. Bagaturov.*

with the Department of Ornithology and Herpetology of the Zoological Institute of the Russian Academy of Sciences.

Leningrad Zoo also works with cooperative *in situ* programs for the reintroduction of the regionally threatened Great crested newt (*Triturus cristatus*). The Moscow Zoo and institutions from the Republic of Georgia support CBPs for the endangered, Caucasian parsley frog (*Pelodytes caucasicus*), and the breeding and rehabitation of other anuran and Caudata species, including *N. kaiseri*, as well as *Megophrys nasutus*, *Tylototriton* spp., and *Cynops* spp. (M. Bagaturov, pers. comm.)

Exhibition design for amphibians (Kreger and Mench 1995; Swanagan 2000) has not received a high research priority (Hurme et al. 2003; Quiguango-Ubillús and Coloma 2008). Amphibian CBPs offer new possibilities for the scope of amphibian displays through using critically endangered species as examples of both amphibian biology and of conservation needs. The Internet is ideally suited to exchanging the information needed to create the most effective displays for threatened species.

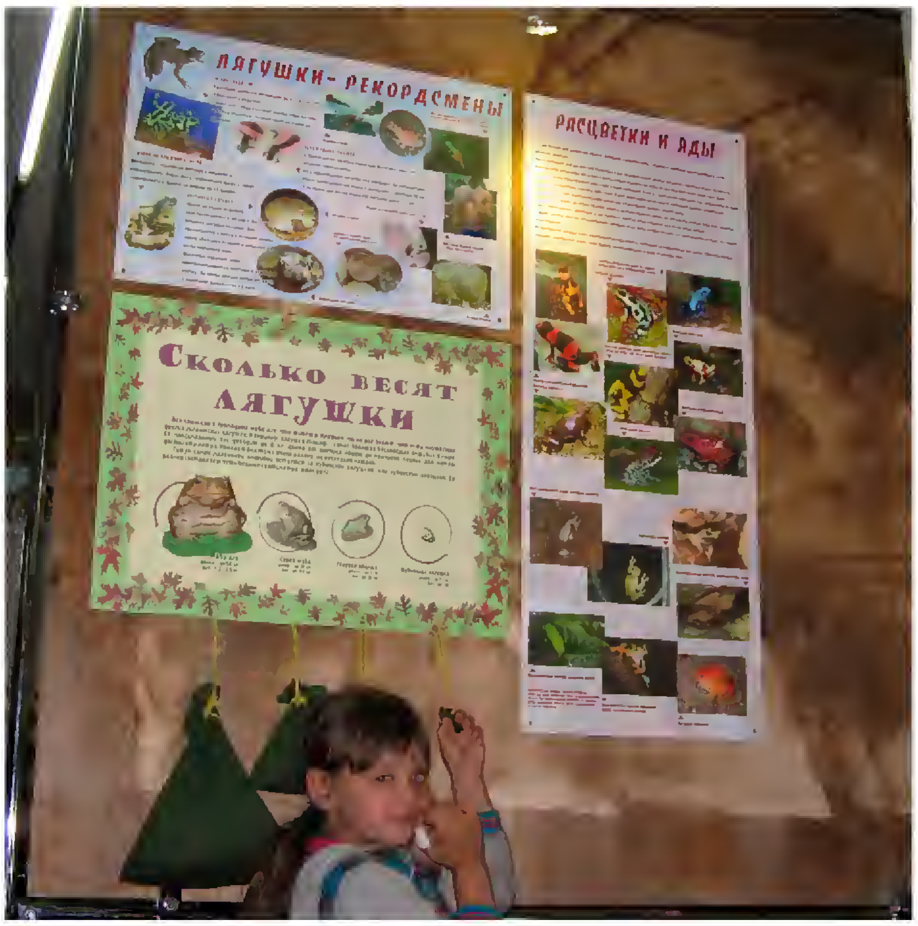


Figure 6. Visitor experience. An interactive educational amphibian exhibit at St. Petersburg Zoo, Russian Federation, not only informs, but also provides tactility to increase fun and experience retention. *Image by Mikhail Bagaturov.*

The exhibition of amphibians arranged in some zoos (e.g., amphibian exhibition in Leningrad Zoo consists of over 30 species of Caudata and Anuran species) accompanied by information desks displaying their biology, reproduction, decline, and how the public may contribute to their conservation. Terraria with amphibians that are decorated in a natural way serve not only the role of attractive exhibitions for visitors but also to display the amphibian's natural habitat (Bagaturov 2011a, b). These and other educational materials make major contributions to the conservation conscience of the zoo's visitors, especially with children.

Direct academic supervision can be very beneficial to amphibian CBPs. Nordens Ark, Sweden, has maintained a foundation that supports amphibian CBPs of threatened species as part of a progressive scientific society with close contacts to universities. Nordens Ark also appointed an academic conservation biologist as scientific leader so that science could inform, management, and implement successful strategies. This initiative has resulted in successful CBPs including reintroduction for the Green toad (*Pseudepidalea viridis*) and the Fire-bellied toad (*Bombina bombina*). Research projects that include undergraduate students from neighboring universities are also proving popular by providing students with a direct, hand's on approach to supporting conservation (Innes 2006).

There are considerable cultural, intellectual, and funding benefits from collaborations for amphibian research between zoos and other institutions, including increased animal welfare, scientific status, conservation commitment, display, and education (Benirschke 1996). Broad cultural collaborations can also increase the impact of exhibitions and educational programs, funding opportunities, as well as providing mutually beneficial intellectual scrutiny and stimulation (Benirschke 1996). Funding bodies can encourage the promotion of projects for both education and the inspiration of future scientists and conservationists (Anderson et al. 2008). CBPs with amphibians have provided many successful research collaborations between zoos, universities, and other entities. For examples, Chester Zoo has many valuable international research collaborations in their CBPs (Chester Zoo 2010).

Collaborations between zoos and private collectors offer a major opportunity to increase the conservation support for many threatened amphibians (Hassapakis 1997). The numbers of species successfully reproduced by private breeders far outweighs those in zoos, and many popular species are now semi-domesticated, including threatened species of anurans and salamanders (Janzen 2010). Caecilians have received less attention, although several aquatic species are bred by private collectors and some zoos (Riga Zoo). Durrell Wildlife Conservation Trust has been involved in a successful joint project with private breeders for the conservation of the Sardinian brook salamander (*Euproctus platycephalus*) using husbandry guidelines developed from private experience. Similarly, the husbandry guidelines for the two critically endangered Iranian newts, the Kurdistan newt (*Neurergus microspilotus*; Browne et al. 2009) and Loristan newt (*N. kaiseri*), were largely developed through the experience of private breeders. Many other species, including some now successfully kept in zoos, these examples of CBPs were formerly bred and distributed via private researchers. Consequently, it is important to not underestimate the contribution of private keepers to amphibian CBP's and to encourage collaboration with private keepers and their organizations wherever possible.

Anderson et al. (2010) conducted a 57-part questionnaire with 210 professionals at AZA zoos and aquariums that were involved in research programs. Support from the chief executive officer and specialized personnel employed to conduct scientific programs were judged as the two most important factors contributing to success. Successful collaboration between zoos and academic institutions required recognition of their different research emphasis. Zoos tend to focus research on animal welfare, conservation, display, and education, while academic institutions focus on description, experimentation, modeling, and specific aspects of animal biology and behavior. Mainly referring to mammals and birds, Fernandez and Timberlake (2008) showed that the main fields of collaboration between zoos and universities were the control and analysis of behavior, conservation and propagation of species, and the education of students and the general public. The latter two are particularly important to amphibian CBPs.

Formal collaboration between institutions can be established by Memorandums of Understanding (MOU), and these should clarify objectives, outcomes, responsibilities, finances, and authorship (Fernandez and Timberlake 2008; Anderson et al. 2010). Innes (2006) considered that many zoos needed an improved communication network between direct research outcomes and animal management.

Scientific knowledge generated from minimally invasive research is more likely to make its way into zoo husbandry and veterinary procedures and provide favorable publicity. Minimally invasive practices can lead to the development of innovative research methods that expand rather than restrict research potential. For instance, noninvasive molecular techniques improve our knowledge of population genetics (Moritz 2008), and assays of hormones improve reproduction and health (Goncharov et al. 1989; Browne et al. 2006; Iimori et al. 2005). Similarly, information systems and databases for amphibian conservation provide the opportunity for extensive analysis of existing data (Melbourne and Hastings 2008), and noninvasive methods such as ultrasound, X-ray, thermal, and photographic digital imaging can address many unsolved research questions. For instance, Nashville Zoo at Grassmere is using ultrasound to determine the reproductive status of the American giant salamander (*C. alleganiensis*) in both their *ex situ* and *in situ* conservation program (D. McGinnity pers. comm.).

Conclusions

Conservation resources for amphibians in many zoos are still largely devoted to display and education and not translated into significant conservation outcomes for specific threatened species. Greater support for conservation can be achieved by zoos also adopting CBPs for threatened amphibian species. Amphibian CBPs and research in zoos can include both *in situ* and *ex situ* components of and preferably should be conducted in concert with in range institutions and programs. Amphibians are ideal subjects for zoo-based research because of the economical provision of their facilities and husbandry and their relatively low maintenance under a variety of research and display conditions. Direct benefits to zoos of amphibian CBPs include the ability to maintain genetically significant numbers, the provision of competent individuals for rehabitation, supplementation, or translocation, the relatively low cost of amphibian research, education, and display, and opportunities for increased outreach and collaboration.

The primary goals of amphibian research in zoos are improved husbandry, health, reproduction, and the perpetuation of genetic variation. Zoos can also provide amphibians to other institutions, such as universities, for conservation-based studies. Research is particularly productive when integrated into CBPs with species that are novel to husbandry, which can then provide significant scientific discoveries. These activities can strengthen all segments of the conservation network between zoos, captive breeding populations, field research, and habitat preservation.

A scientific program with administrative support and dedicated facilities will attract qualified candidates for research and education positions. To maximize the productivity and quality of "Zoo-based amphibian research and conservation" qualified researchers with academic affiliations should be employed. Within this framework, institutions can design a science-based management structure for research that is tailored to their institutional capacity and amphibian collection (Hutchins 1988).

Amphibian research in zoos offers opportunities to form research collaborations with universities and other institutions, both regionally and internationally (Fernandez and Timberlake 2008; Lawson et al. 2008). Through their capacity for fund raising, grants, organizational capacity, and academic affiliations, zoos can develop projects of international stature through CBPs for threatened species (Lawson et al. 2008; Reid et al. 2008). Amphibian research in zoos can offer students and young conservation scientist's attractive opportunities to participate directly in amphibian welfare and to directly contribute to amphibian conservation through research projects of short duration (Kleiman 1996).

Acknowledgments.—This work was supported by core funding from the Flemish Government. Special thanks to Prof. Thomas Ziegler for his comments on this manuscript.

Literature cited

Allentoft ME, O'Brien J. 2010. Global amphibian declines, loss of genetic diversity and fitness: a review. *Diversity* 2(1):47-71.

Amphibian Ark. 2010. *Geocrinia* programs at Perth Zoo. *AArk Newsletter*, 13 (December 2010). [Online]. Available: http://www.amphibianark.org/Newsletters/pdf_newsletters/AArk%20Newsletter%2013.pdf [Accessed: 13 October 2011].

AmphibiaWeb. 2011 (22 January 2009). Worldwide amphibian declines: How big is the problem, what are the causes and what can be done? [Online]. Available: http://amphibiaweb.org/declines/declines.html [Accessed: 13 October 2011].

Anderson US, Bloomsmith MA, Maple TL. 2010. Factors facilitating research: a survey of zoo and aquarium professionals. *Zoo Biology* 29(6):663-675.

Anderson US, Kelling AS, Maple TL. 2008. Twenty-five years of Zoo Biology: a publication analysis. *Zoo Biology* 27(6):444-457.

Andrén C, Blomqvist L, Lindén M. 2011. Nordens Ark – a university conservation field station. *International Zoo Yearbook* 45(1):1-12.

Antwis RE, Browne RK. 2009. Ultraviolet radiation and Vitamin D_3 in amphibian health, behaviour, diet and conservation. *Comparative Biochemistry Physiology – Part A: Molecular & Integrative Physiology* 154(2):184-190.

Araki H, Cooper B, Blouin MS. 2007. Genetic effects of captive breeding cause a rapid, cumulative fitness decline in the wild. *Science* 318(5847):100-103.

Bagaturov M. 2011. Some notes on keeping *Theloderma*. *Herp Nation Magazine* 2(1):34-39.

Bagaturov M. 2011. Haltung und Nachzucht von Moosfroschen der Gattung *Theloderma* – einige Anmerkungen aus der Praxis. *DRACO* 46(12):29-34.

Baker A. 2007. Animal ambassadors: an analysis of the effectiveness and conservation impact of *ex situ* breeding efforts. In *Zoos in the 21st Century: catalysts for conservation?* Editors, A Zimmerman, M Hatchwell, C West, R Lattis. Cambridge University Press, Cambridge, UK. 388 p. 139-154.

Benirschke K. 1997. The need for multidisciplinary research units in the zoo. In *Wild Mammals in Captivity: principles and techniques*. Editors, DG Kleiman, ME Allen, KV Thompson, S. Lumpkin, H. Harris. University of Chicago Press, Chicago, Illinois and London. 656 p. 537-544.

Bradshaw CJA, Sodhi NS, Brook BW. 2009. Tropical turmoil: a biodiversity tragedy in progress. *Frontiers in Ecology and the Environment* 7(2):79-87.

Browne RK, Figiel C. 2011. Cryopreservation in amphibians. In *Cryopreservation of Aquatic Species*. Volume 8. Editors, Dr. T. Tiersch, Dr. P. Mazik. World Aquaculture Society, Baton Rouge, Louisiana. 1003 p. 346-365.

Browne RK, Alford R, Mendelson J, Zippel K. 2010. Spotlight on amphibian research in zoos. *AArk Newsletter*, No. 12. (September 2010). [Online]. Available: http://www.amphibianark.org/Newsletters/pdf_newsletters/Amphibian%20Ark%20News%2012.pdf [Accessed: 13 October 2011].

Browne RK, Li H, Robinson H, Uteshev VK, Shishova NR, McGinnity D, Nofs S, Figiel CR, Mansour N, Lloyd RE, Agnew D, Carleton CL, Wu M, Gakhova EN. 2011. Reptile and amphibian conservation through gene banking and other reproduction technologies. *Russian Journal of Herpetology* 18(3):165-174.

Browne RK, Mendelson JR, McGregor RG, Alford R, Zippel K, Pereboom JJM. (Editors). 2009. Edition 1. *Amphibian Conservation Research Guide*. IUCN/ASG/Amphibian Ark. 40 p. [Online]. Available: http://aark.portal.isis.org/ResearchGuide/ACRG/Amphibian%20Conservation%20Research%20Guide.pdf [Accessed: 13 October 2011].

Browne RK, Pereboom Z. 2009. Lake Oku clawed frog rescued. *AArk Newsletter*, No. 6 (March 2009). [Online]. Available: http://www.amphibianark.org/Newsletters/amphibian_ark_news_6.htm [Accessed: 13 October 2011].

Browne RK, Rastegar-Pouyani N, Sharifi M. 2009. Taxon management plan for *Neurergus microspilotus*. [Online]. Available: http://www.amphibianark.org/pdf/Husbandry/Neurergus%20microspilotus%20Taxon%20Management%20Plan.pdf [Accessed: 13 October 2011].

Browne RK, Seratt J, Vance C, Kouba A. 2006a. Hormonal induction with priming and in vitro fertilisation increases egg numbers and quality in the Wyoming toad (*Bufo baxteri*). *Reproductive Biology Endocrinology* 4:34(1-11).

Browne RK, Seratt J, Li H, Kouba A. 2006b. Progesterone improves the number and quality of hormonally induced fowler toad (*Bufo fowleri*) oocytes. *Reproductive Biology and Endocrinology* 4:3(1-7).

Browne RK, Zippel K. 2007. Reproduction and larval rearing. In *Use of Amphibians in Research, Laboratory, or Classroom Settings*. Institute for Laboratory Animal Research 48(3):214-234. [Online]. Available: http://delsold.nas.edu/ilar_n/ilarjournal/48_3/pdfs/4803Browne1.pdf [Accessed: 13 October 2011].

Burggren WW, Warburton S. 2007. Amphibians as animal models for laboratory research of physiology. In *Use of Amphibians in Research, Laboratory, or Classroom Settings*. Institute for Laboratory Animal Research 48(3):188-202. [Online]. Available: http://delsold.nas.edu/ilar_n/ilarjournal/48_3/pdfs/4803Burggren.pdf [Accessed: 13 October 2011].

The Center for North American Herpetology (CNAH). *North American Introduced Exotic Species Checklist.* [Online]. Available: http://www.cnah.org/ex_nameslist.asp [Accessed: 23 October 2011].

Chester Zoo. 2010. Research. [Online]. Available: http://www.chesterzoo.org/conservation-and-research [Accessed: 18 October 2011].

Daszak P, Berger L, Cunningham AA, Hyatt AD, Green DE, Speare R. 1999. Emerging infectious diseases and amphibian population declines. *Emerging Infectious Diseases* 5(6):735-748.

Davic RD, Welsh HH Jr. 2004. On the ecological role of salamanders. *Annual Review of Ecology, Evolution, and Systematics* 35:405-434.

Dodd CK, Seigel RA. 1991. Relocation, repatriation, and translocation of amphibians and reptiles: Are they conservation strategies that work? *Herpetologica* 47(3):336-350.

EAZA. 2009. EAZA Strategy 2009-2012. *About EAZA, Our mission*. [Online]. Available: http://www.eaza.net/about/Pages/Introduction.aspx [Accessed: 13 October 2011].

EDGE. 2010. EDGE, Evolutionary Distinct and Globally Endangered, Zoological Society of London. [Online]. Available: http://www.edgeofexistence.org/ [Accessed: 13 October 2011].

Fernandez EJ, Timberlake W. 2008. Mutual benefits of research collaborations between zoos and academic institutions. *Zoo Biology* 27(6):470-487.

Foden W, Mace G, Vié JC, Angulo A, Butchart S, DeVantier L, Dublin H, Gutsche A, Stuart S, Turak E. 2008. Species susceptibility to climate change impacts. In *The 2008 Review of the IUCN Red List of Threatened Species*. Editors, JC Vié, C Hilton-Taylor, SN Stuart. IUCN Gland, Switzerland. [Online]. 1-11. Available: http://cmsdata.iucn.org/downloads/species_susceptibility_to_climate_change_impacts_1.pdf [Accessed: 13 October 2011].

Furrer S, Corredor G. 2008. Conservation of threatened amphibians in Valle des Cauca, Colombia: a cooperative project between Cali Zoological Foundation, Colombia, and Zoo Zürich, Switzerland. *International Zoo Yearbook* 42(1):158-164.

Garcia G, Cunningham AA, Horton DL, Garner TWJ, Hyatt A, Hengstberger S, Lopez J, Ogrodowczyk A, Fenton C, Fa JE. 2007. Mountain chickens *Leptodactylus fallax* and sympatric amphibians appear to be disease free on Montserrat. *Oryx* 41(3):398-401.

Gascon C, Collins JP, Moore RD, Church DR, McKay JE, Mendelson Jr III. (Editors). 2007. *Amphibian Conservation Action Plan*. IUCN/SSC Amphibian Specialist Group, Gland, Switzerland and Cambridge, UK. 64 p. [Online]. Available: http://amphibiaweb.org/declines/acap.pdf [Accessed: 13 October 2011].

Germano JM, Bishop PJ. 2009. Suitability of reptiles and amphibians for translocation. *Conservation Biology* 23(1):7-15.

Goncharov BF, Shubravy OI, Serbinova IA, Uteshev VK. 1989. The USSR program for breeding amphibians, including rare and endangered species. *International Zoo Yearbook* 28:10-21.

Griffiths RA, Pavajeau L. 2008. Captive breeding, reintroduction, and the conservation of amphibians. *Conservation Biology* 22(4):852-861.

Hassapakis C. 1997. The worldwide conservation breeding of threatened reptilian species In *The Biology, Husbandry, and Health Care of Reptiles*. Volume 1 (*The Biology of Reptiles*) [ISBN: 0-793-80501-5]. 392 p.; Volume 2 (*The Husbandry of Reptiles*) [ISBN: 0-793-80502-3]. 230 p.; and Volume 3 (*The Health Care of Reptiles*) [ISBN: 0-793-80503-1]. 446 p. Nine Appendices. Editor, LJ Ackerman. TFH Publications, Neptune, New Jersey. [ISBN: 0-793-80504-X, for all three volumes]. 1068 p. 235-271, 963-972.

Hutchins M, Thompson SD. 2008. Zoo and aquarium research: priority setting for the coming decades. *Zoo Biology* 27(6):488-497.

Hutchins M, Conway WM. 1995. Beyond Noah's Ark: the evolving zoological parks and aquariums in field conservation. *International Zoo Yearbook* 34(1):117-130.

Hutchins M. 1988. On the design of zoo research programs. *International Zoo Yearbook* 27(1):9-19.

Hurme K, Gonzalez K, Halvorsen M, Foster B, Moore D. 2003. Environmental enrichment for dendrobatid frogs. *Journal of Applied Animal Welfare Science* 6(4):285-299.

Iimori E, D'Occhio MJ, Lisle AT, Johnston SD. 2005. Testosterone secretion and pharmacological spermatozoal recovery in the cane toad. *Animal Reproduction Science* 90(1-2):163-173.

Innes J. 2006. *Scholarly Communication and Knowledge Management in American Zoos*. Ph.D. Theses. Graduate School of Computer and Information Sciences, Nova Southeastern University. 310 p. [Online]. Available: http://gradworks.umi.com/32/34/3234986.html [Accessed: 13 October 2011].

IUCN. 2010. *The IUCN Red List of Threatened Species*. [Online]. Available: http://www.iucnredlist.org/ [Accessed: 14 October 2011].

Jansen BJ, Spink A. 2006. How are we searching the World Wide Web? A comparison of nine search engine transaction logs. *Information Processing and Management* 42(1):248-263.

Janzen P. 2010. Cooperative breeding of amphibians by zoos and private herpetologists in the "German speaking area." *AArk Newsletter* (March 2010). [Online]. Available: http://www.amphibianark.org/Newsletters/pdf_newsletters/amphibian%20ark%20news%2010.pdf [Accessed: 14 October 2011].

Kaurova SA, Chekurova NR, Melnikova EV, Uteshev VK, Gakhova EN. 1996. Cryopreservation of frog *Rana temporaria* sperm without loss of fertilizing capacity. In *Genetic Resource Conservation (Proceedings of XIV Working Meet-*

ing, May 13-15, 1996, Pushchino, Russian Federation). 106-108.

King JD, Muhlbauer MC, James A. 2010. Radiographic diagnosis of metabolic bone disease in captive bred mountain chicken frogs (*Leptodactylus fallax*). *Zoo Biology 30(3):254-259.*

Kreger MD, Mench JA. 1995. Visitor–animal interactions at the zoo. *Anthrozoös: a multidisciplinary journal of the interactions of people & animals* 8(3):143-158.

Lawson DP, Ogden J, Snyder RJ. 2008. Maximizing the contribution of science in zoos and aquariums: organizational models and perceptions. *Zoo Biology* 27(6):458-469.

Lees CM, Wilcken J. 2009. Sustaining the Ark: the challenges faced by zoos in maintaining viable populations. *International Zoo Yearbook* 43(1):6-18.

Li H, Vaughan MJ, Browne RK. 2009. A complex enrichment diet improves growth and health in the endangered Wyoming toad (*Bufo baxteri*). *Zoo Biology* 28(3):197-213.

Lips KR, Reeve JD, Witters LR. 2003. Ecological traits predicting amphibian population declines in Central America. *Conservation Biology* 17(4):1078-1088.

Lötters S. 2008. Afrotropical amphibians in zoos and aquariums: Will they be on the ark? *International Zoo Yearbook* 42(1):136-142.

Mansour N, Lahnsteiner F, Patzner RA. 2011. Collection of gametes from live axolotl, *Ambystoma mexicanum*, and standardization of in vitro fertilization. *Theriogenology* 75(2):354-361.

Martin L, Morton MN, Hilton GM, Young RP, Garcia G, Cunningham AA, James A, Gray G, Mendes S. (Editors). 2007. *A Species Action Plan for the Montserrat Mountain Chicken Leptodactylus fallax*. Department of Environment, Montserrat. 63 p. [Online]. Available: http://www.durrell.org/library/Document/Montserrat_mountain_chicken_action_plan.pdf [Accessed: 13 October 2011].

McCallum ML. 2007. Amphibian decline or extinction? current declines dwarf background extinction rate. *Journal of Herpetology* 41(3):483-491.

McDonald M, Sayre T. 2008. Climate change. AmphibiaWeb. [Online]. Available: http://amphibiaweb.org/declines/ClimateChange.html [Accessed: 14 October 2011].

Melbourne BA, Hastings A. 2008. Extinction risk depends strongly on factors contributing to stochasticity. *Nature* 454:100-103.

Mohneke M, Ródel MO. 2009. Declining amphibian populations and possible ecological consequences – a review. *Salamandra* 45(4):203-210.

Moritz C. 2008. Applications of mitochondrial DNA analysis in conservation: a critical review. *Molecular Ecology* 3(4):401-411.

Michigan State University. 2010 (04 August 2010). Preserving sperm vital to saving 'snot otter' salamanders. *ScienceDaily*. [Online]. Available: http://www.sciencedaily.com/releases/2010/08/100804110210.htm [Accessed: 14 October 2011].

National Geographic. 2010. "Snot Otter" sperm to save giant salamander? [Online]. Available: http://news.nationalgeographic.com/news/2010/08/100820-hellbenders-snot-otters-sperm-amphibians-science-environment/ [Accessed: 14 October 2011].

National Biological Information Infrastructure (NBII), USA. 2011. Invasive amphibians and reptiles. [Online]. Available: http://www.nbii.gov/portal/server.pt/community/amphibians_and_reptiles/932 [Accessed: 23 October 2011]. NOTE: In the 2012 President's Budget Request, the National Biological Information Infrastructure (NBII) is terminated. As a result, all resources, databases, tools, and applications within this web site will be removed on January 15, 2012. For more information, please refer to the NBII Program Termination [http://www.nbii.gov/portal/server.pt/community/termination_of_nbii_program/2057] page.

Newman MEJ. 2001. The structure of scientific collaboration networks. *Proceedings of the National Academy of Sciences USA* 98(2):404-409.

Nguyen TQ, Dang TT, Pham CT, Nguyen TT, Ziegler T. 2009. Amphibian breeding station in Hanoi: a trial model for linking conservation and research with sustainable use. *Froglog* 91:12-15.

Obringer AR, O'Brien JK, Saunders RL, Yamamoto K, Kikuyama S, Roth TL. 2000. Characterization of the spermiation response, luteinizing hormone release and sperm quality in the American toad (*Bufo americanus*) and the endangered Wyoming toad (*Bufo baxteri*). *Reproduction, Fertility and Development* 12(2):51-58.

Olsen GM, Zimmerman A, Bos N. 2008. Scientific collaboration on the Internet. CM Digital Library. [Online]. Available: http://dl.acm.org/citation.cfm?id=1522423 [Accessed: 14 October 2011].

Pessier AP, Mendelson Jr. (Editors). 2010. *A Manual for Control of Infectious Diseases in Amphibian Survival Assurance Colonies and Reintroduction Programs*. IUCN/SSC Conservation Breeding Specialist Group, Apple Valley, Minnesota, USA. 221 p.

Pessier AP. 2008. Management of disease as a threat to amphibian conservation. *International Zoo Yearbook* 42(1):30-39.

Quiguango-Ubillús A, Coloma LA. 2008. Notes on the behaviour, communication and reproduction in captive *Hyloxalus toachi* (Anura: Dendrobatidae), an endangered Ecuadorian frog. *International Zoo Yearbook* 42(1):78-89.

Read D, Scarparolo D. 2010. *Geocrinia* programs at Perth Zoo. *AArk Newsletter*, No. 13. [Online]. Available: http://www.amphibianark.org/Newsletters/pdf_newsletters/AArk%20Newsletter%2013.pdf [Accessed: 14 October 2011].

Reid GMcG, Macdonald AA, Fidgett AL, Hiddinga B. Leus K. 2008. Developing the research potential of zoos and aquaria. In *The EAZA Research Strategy*. EAZA Executive Office, Amsterdam. [Online]. Available: http://www.eaza.net/about/Documents/EAZA_Research_Strategy_2008.pdf [Accessed: 14 October 2011].

Rohr JR, Schotthoefer AM, Raffel TR, Carrick HJ, Halstead N, Hoverman JT, Johnson CM, Johnson LB, Lieske C, Piwoni MD, Schoff PK, Beasley VR. 2008. Agrochemicals increase trematode infections in a declining amphibian species. *Nature* 455:1235-1239.

Shishova NR, Uteshev VK, Kaurova SA, Browne RK, Gakhova EN. 2010. Cryopreservation of hormonally induced sperm for the conservation of threatened amphibians with *Rana temporaria* as a model research species. *Theriogenology* 75(2):220-232.

Skerratt LF, Berger L, Speare R, Cashins S, McDonald KR, Phillott AD, Hines HB, Kenyon N. 2007. Spread of chytridiomycosis has caused the rapid global decline and extinction of frogs. *EcoHealth* 4(2):125-134.

Sodhi NS, Bickford D, Diesmos AC, Lee TM, Koh LP, Brook BW, Cekercioglu CH, Bradshore CJA. 2008. Measuring the meltdown: drivers of global amphibian extinction and decline. *PLoS ONE* 3(2):e1636(1-8).

Soulé M, Gilpin M, Conway W, Foose T. 1986. The Millenium Ark: How long a voyage, how many staterooms, how many passengers? *Zoo Biology* 5(2):101-113.

Swanagan JS. 2000. Factors influencing zoo visitors' conservation attitudes and behavior. *The Journal of Environmental Education* 31(4):26-31.

Trudeau VL, Somoza GM, Natale GS, Pauli B, Wignall J, Jackman P, Doe K, Schueler FW. 2010. Hormonal induction of spawning in 4 species of frogs by coinjection with a gonadotropin-releasing hormone agonist and a dopamine antagonist. *Reproductive Biology and Endocrinology* 8:36(1-9).

UNEP-WCMC. 2009. *Internet Searches to Monitor Wildlife Trade*. SRG 50/10. UNEP-WCMC, Cambridge. 13 p. [Online]. Available: http://www.cites.org/common/cop/15/inf/C15i-66.pdf [Accessed: 18 October 2011].

United Nations. 2004. World population growth. [Online]. Available: http://www.un.org/esa/population/publications/sixbillion/sixbilpart1.pdf [Accessed: 14 October 2011].

WAZA. 2009. *Zoos and Aquariums of the World*. [Online]. Available: http://www.waza.org/en/site/zoos-aquariums [Accessed: 14 October 2011].

WAZA. 2005. *Building a Future for Wildlife: the world zoo and aquarium conservation strategy*. World Association of Zoos and Aquariums, Bern, Switzerland. 72 p. [Online]. Available: http://www.waza.org/files/webcontent/documents/wzacs/wzacs-en.pdf [Accessed: 14 October 2011].

Wright, SJ, Muller-Landau, HC. 2006a. The future of tropical forest species. *Biotropica* 38(3):287-301.

Wright, SJ, Muller-Landau, HC. 2006b. The uncertain future of tropical forest species. *Biotropica* 38(4):443-445.

Zippel K, Johnson K, Gagliardo R, Gibson R, McFadden M, Browne R, Martinez C, Townsend E. 2011. The Amphibian Ark: a global community for *ex situ* conservation of amphibians. *Herpetological Conservation & Biology*, in press.

Zippel K. 2009. Zoos play a vital role in amphibian conservation. AmphibiaWeb. [Online]. Available: http://amphibiaweb.org/declines/zoo/index.html [Accessed: 14 October 2011].

Ziegler T. 2007. Field surveys and collection management as basis for herpetodiversity research and nature conservation in Vietnam. In *Ho Chi Minh City People's Committee, Viet Nam Union of Science and Technology Associations, Colivam, PTC (Hrsg.): Development of Hochiminh City Museum of Natural History*. Proceedings International Conference, Ho Chi Minh City, Sept. 12-15. 230-248.

Ziegler T. 2010. Amphibian and reptilian diversity research, conservation and breeding projects in Vietnam. In *Building a Future for Wildlife: zoos and aquariums committed to biodiversity conservation*. Editors, G. Dick, M. Gusset. WAZA Executive Office, Gland, Switzerland. 117-122.

Ziegler T, Vu TN. 2009. Ten years of herpetodiversity research in Phong Nha – Ke Bang National Park, central Vietnam. In *Phong Nha – Ke Bang National Park and Cologne Zoo, 10 years of cooperation (1999-2009)*. Editors, VT Vo, TD Nguyen, NK Dang, TY Pham, B Quang. 103-124.

Ziegler T, Nguyen TQ. 2008. The amphibian and reptilian breeding station at Hanoi. In *Amphibian and Reptile Breeding*. WAZA Magazine (World Association of Zoos and Aquariums) Nr. 9 (Zoos help sustaining the rich biodiversity of Vietnam). 10-14.

Ziegler T, Dang TT, Nguyen TQ. 2011. Breeding, natural history and diversity research: "*ex situ*" and "*in situ*" Asian amphibian projects of the Cologne Zoo and the Institute of Ecology and Biological Resources. *Proceedings of the Amphibian Conference of the Universiti Malaysia Sarawak*. Kuching, Borneo, in press.

Ziegler T, Ohler A. Vu NT, Le KQ, Nguyen XT, Dinh HT, Bui NTh. 2006. Review of the amphibian and reptile diversity of Phong Nha – Ke Bang National Park and adjacent areas, central Truong Son, Vietnam. In *Herpetologia Bonnensis II*. Editors, M Vences, J Köhler, T Ziegler, W Böhme. Proceedings of the 13th Ordinary General Meeting of the Societas Europaea Herpetologica, Bonn. 247-262.

Received: 26 August 2011
Accepted: 10 October 2011
Published: 30 October 2011

ROBERT BROWNE has worked as an investment manager, builder, design draftsman, video producer, professional photographer and he has now found his true vocation, Conservation Biologist and Collaborative Researcher.

Robert has completed an Honour's degree in Aquaculture at the Key Center for Aquaculture, Australia, and then obtained a Ph.D. (1998) in Conservation Biology from the University of Newcastle, Australia.

Robert's science employment has included consultancy with biotechnology corporations and in response to the global biodiversity conservation crisis has focused on amphibian conservation and sustainability. Working with zoos in Australia, the USA, Europe, and as Research Officer for the IUCN has led Robert to work with collaborative conservation programs in the USA, Peoples Republic of China, Australia, Russian Federation, Islamic Republic of Iran, and Cameroon.

Robert has experience in a wide range of research fields supporting herpetological conservation and environmental sustainability. He has published in the scientific fields of nutrition, pathology, larval growth and development, husbandry, thermobiology, reproduction technologies, and facility design.

Robert's Ph.D. in the late 1990s was seminal to the development of gene banking to preserve genetic diversity of threatened species. Since then his research with reproduction technologies has led to major advances in the use of hormones to promote amphibian reproduction. This was responsible for the first use of artificial fertilization, to produce tadpoles for release, of the critically endangered amphibian, the Wyoming toad (*Bufo baxteri*). These techniques have since been adopted for a number of other critically endangered amphibian species. Robert's recent collaborative work with Nashville Zoo at Grassmere, USA, and international organizations on the North American giant salamander (*Cryptobranchus alleganiensis*), commonly known as the Hellbender, has fostered the development of the first genetically representative gene bank for any amphibian.

KATJA WOLFRAM focused her undergraduate studies on marine biology, zoology, and genetics and graduated with a Diplom in biology at Bremen University, Germany. In her graduation thesis, she addressed population genetics as well as physiology, and genetics, of the respiratory pigment in the Common European cuttlefish *Sepia officinalis*. Currently, she is completing her Ph.D., thesis at Antwerp Zoo's Centre for Research and Conservation (Antwerp, Belgium), researching the genetic background of mate choice in the Eurasian black vulture, *Aegypius monachus*, a species of conservation concern.

MIKHAIL F. BAGATUROV formerly a professional lawyer, was always a wild fauna collector and researcher traveling to the Middle Asia, Caucasus, Crimea, Siberia, Baltic region, Carpathians, and most of the former USSR territories with exception of the Russian Far East. An exotic animal keeper and breeder all his life Mikhail now works at the Leningrad Zoo (Saint Petersburg, Russia) as a zootechnist in the Department of Insectarium and Amphibians.

Mikhail is a member of the Russian Nikolsky's Herpetological Society at Russian Academy of Sciences and has been a terrarium animal keeper for over 30 years (one of the most experienced animal keepers in the former USSR).

In 2009, Mikhail began contributing to programs of study on the biodiversity of herpetofauna in Vietnam under the auspices of the Department of Herpetology, Zoological Institute of the Russian Academy of Sciences, St. Petersburg, Russia (Profs. Profs. Natalia Ananjeva and Nikolai Orlov).

Since 2010, Mikhail has been a member of Conservation Breeding Specialist Group (CBSG), Species Survival Commission (SSC), International Union for Conservation of Nature (IUCN), which is dedicated to saving threatened species by increasing the effectiveness of conservation efforts worldwide.

Since 2011, Mikhail had been a member of IUCN/SSC Amphibian Specialist Group (ASG).

While a large part of Mik's work is with amphibians and reptiles, he is also working on developing techniques for captive management of a variety of invertebrate groups with special focus on Theraphosid spiders (Tarantulas). Mikhail is further working on international programs on invertebrate husbandry and conservation under the guidance of the Terrestrial Invertebrates Advisory Group, European Association of Zoos and Aquariums (TITAG-Europe).

Mikhail has present plans to start a Ph.D. program at the Department of Herpetology, Zoological Institute, Russian Academy of Sciences, with research focusing on the reproductive biology of amphibians.

GERARDO GARCÍA was born in Barcelona (Spain) and has been Head of the Herpetology Department at Durrell Wildlife Conservation Trust, based in Jersey, United Kingdom (UK), since 2003. His herpetological career began at Barcelona Zoo in 1992 becoming involved in the early years of the Recovery Programme for the Mallorcan midwife toad (*Baleaphryne muletensis*) and at the Science Museum of Barcelona (CosmoCaixa) up until 1996, when he moved for work to Thoiry Zoo (Paris, France).

Gerardo's work with amphibians since 1992 has involved captive breeding programs of reptiles and amphibians in several institutions, linking *ex situ* with *in situ* conservation in Jersey (*Rana dalmatina*, *Bufo bufo*), Montserrat/Dominica (*Leptodactylus fallax*), Madagascar (*Erymnochelys madagascariensis*, *Pyxis planicauda*, *Astrochelys yniphora*), Spain (*Alytes obstetricans*, *Rana iberica*), and Mauritius (*Nactus coindemirensis*, *Gongylomorphus fontenayi* sp.). During the last few years he has been involved in various training initiatives for amphibians around the world (France, Germany, Sweden, Spain, South Africa, Mexico, Madagascar, India, Sri Lanka, Colombia, Venezuela, Montserrat, and Dominica), improving the husbandry protocols of captive colonies and diverse *in situ* programs such as the Montserrat mountain chicken frogs, genus *Alytes* and *Rana* in Spain and the amphibians of Jersey.

Gerardo completed a Ph.D. at the Institute of Conservation and Ecology (DICE), University of Kent on the "Ecology, human impact, and conservation of the Madagascan side-necked turtle (*Erymnochelys madagascariensis*) at Ankarafantsika National Park," where he lived for two years during his data collection and field work in Madagascar. Gerardo analyzed his data and began to write his thesis at the Laboratoire des Reptiles et Amphibiens, Muséum d'Histoire Naturelle of Paris, moving to Jersey in 2001.

Gerardo has been actively involved in the European Association of Zoos and Aquariums (EAZA) as chair of the Amphibian Taxon Advisory Group (ATAG) and vice-chair for the Reptile Taxon Advisory Group (RTAG). His major goal is to bring *in situ* conservation and research for these programs into the core activities of the EAZA. Gerardo was actively involved in the development of the amphibian campaign for the *Year of the Frog 2008* and co-directed the first amphibian conservation courses in Europe for Zoos and Aquariums in 2006 and 2008.

Gerardo also takes a great interest in raising the profile of the herpetological programs within both specialist groups and the general public. In his spare time, he assists affiliate zoological institutions in the development of their animal collections, design exhibits, and off show facilities for reptiles and amphibians, and in the development of new conservation programs.

ZJEF J. J. M. PEREBOOM is head of the Center for Research and Conservation and coordinator of Behavioral Research, Royal Zoological Society of Antwerp, Antwerp, Belgium. His research interests include behavioral and evolutionary ecology of primates, birds, and social insects, and the ethology of zoo animals with a link to conservation biology and animal welfare. Zjef is particularly interested in sexual selection processes and how they affect e.g., captive breeding programmes in particular, and population management measures in general.

Copyright: © 2012 Wildenhues et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Amphibian & Reptile Conservation 5(3):15-28.

Husbandry, captive breeding, larval development and stages of the Malayan horned frog *Megophrys nasuta* (Schlegel, 1858) (Amphibia: Anura: Megophryidae)

[1,3]MARLEN WILDENHUES, [1]ANNA RAUHAUS, [1,4]RIKE BACH, [1]DETLEF KARBE, [1]KARIN VAN DER STRAETEN, [2,5]STEFAN T. HERTWIG, AND [1,6]THOMAS ZIEGLER

[1]*Cologne Zoo, Cologne, GERMANY* [2]*Naturhistorisches Museum der Burgergemeinde Bern, Berne, SWITZERLAND*

***Abstract*.—We report long-term experience with the successful keeping and breeding of *Megophrys nasuta* at the Cologne Zoo's Amphibian Breeding Unit and compare data with other breeding reports. In addition, we document the development and morphology of different larval stages of *M. nasuta*. Diagnostic morphological characters are provided for Gosner (1960) larval stages 18-22 and 25-46. Ovipositions were not seasonal and took place after a drier phase in the terrarium followed by intensive spraying to simulate the natural rain period. The larvae hatched about one week after egg deposition. The characteristic funnel-shaped oral disc became discernible about two weeks after egg deposition at Gosner stage 21 and degenerated at Gosner stage 42. The mean total developmental time observed for *M. nasuta* was 2.5-3.5 months. Larvae developed faster at higher temperatures and lower densities. The triangular projections at the upper eyelids, which are characteristic for advanced terrestrial stages, began to develop two or three weeks after completion of metamorphosis.**

Key words. Anura, Megophryidae, *Megophrys nasuta*, husbandry, captive breeding, development, larval stages

Citation: Wildenhues M, Rauhaus A, Bach R, Karbe D, Van der Straeten K, Hartwig ST, Ziegler T. 2012. Husbandry, captive breeding, larval development and stages of the Malayan horned frog *Megophrys nasuta* (Schlegel, 1858) (Amphibia: Anura: Megophryidae). *Amphibian & Reptile Conservation* 5(3):15-28(e43).

Introduction

The Malayan horned frog, *Megophrys nasuta*, was originally described by Schlegel (1858). For some time this taxon was considered to be a subspecies of *M. monticola*, Kuhl and Van Hasselt, 1822 (e.g., Inger 1954, 1966), but is now considered to be a synonym of *M. montana*, Kuhl and Van Hasselt, 1822 (Frost 2011). The genus *Megophrys* includes the following four species besides *M. nasuta*: *M. kobayashii* Malkmus and Matsui, 1997, *M. ligayae* Taylor, 1920, *M. montana* Kuhl and Van Hasselt, 1822, and *M. stejnegeri* Taylor, 1920 (Frost 2011). The recently described *M. damrei* Mahony, 2011 and *M. takensis* Mahony, 2011 were allocated to the genus *Xenophrys* by Frost (2011), which was considered to be a junior synonym of *Megophrys* by Mahony (2011).

Megophrys nasuta is known to occur in Sumatra, Borneo, and Malaysia; records from Thailand to the Sunda Shelf may belong to other species (Frost 2011). Diagnostic characters of species are presence of a dermal rostral appendage, a triangular projection on the upper eyelid, two pairs of parallel, longitudinal, dorsolateral folds continuous between head and groin, and its large size. Females may reach a snout-vent length of 160 mm, and smaller males 105 mm (Inger 1966; Manthey and Grossmann 1997; Malkmus et al. 2002). The head appendages and projections together with the cryptic coloration serve as phytomimesis in the leaf litter of the forest floor. *Megophrys nasuta* is regularly encountered in intact lowland and submontane rainforest up to an elevation of 1,300 m, mostly in the vicinity of forest streams. Adults are terrestrial and nocturnal and tadpoles are funnel-mouthed surface dwellers in clear forest streams (Malkmus et al. 2002; van Dijk et al. 2004).

The IUCN lists *M. nasuta* as a taxon of Least Concern because of its wide distribution range and presumed large population size. Habitat loss and fragmentation are among the major known threats to *M. nasuta* and harvesting for national and international pet trade may also threaten some populations (van Dijk et al. 2004). Because of the global amphibian crisis, including the possibility that amphibian chytrid fungus (*Batrachochytrium dendrobatidis*) may cause extinction of local populations or species (e.g., Berger et al. 1998; Briggs et al. 2005; Mendelson et al. 2006), captive breeding programs have become crucial tools for amphibian conservation (Griffiths and Pavajeau 2008; McGregor Reid and Zippel 2008; Browne et al. 2011; Ziegler et al. 2011; Zippel et al. 2011).

Correspondence. *Email:* [3]*marlen.wildenhues@gmx.de;* [4]*RikeBach@web.de;* [5]*stefan.hertwig@nmbe.ch;* [6]*ziegler@koelnerzoo.de* (Corresponding author).

Megophrys nasuta is rarely bred in captivity (Schmidt 1976, 1977; Schmidt and Wicker 1977; Schwanz 1977; Rogner 1980; Pfeuffer 1989; Anonymus 1994; v. d. Nieuwenhuizen 2001a, b), and because of increasing threats to this and other *Megophrys* species, here we present our long-term experience with the successful husbandry of *M. nasuta* at the Cologne Zoo (see also van der Straeten et al. 2007; Ziegler et al. 2008). In addition, we present the first staging table for *M. nasuta* or for any *Megophrys* species.

Materials and methods

Collection, identification and abbreviations

When beginning our breeding program for *M. nasuta* at the Cologne Zoo, Germany, in 2005 we had access to three males and two females obtained from the pet trade. According to the trader, these frogs were from the federal states of Pahang or Perak, Malaysia. Breeding and rearing was achieved between 2006 and 2009.

For verification of species, at various times during our breeding program deceased specimens were fixed in 40-60% ethanol, preserved in 70% ethanol and subsequently deposited in the herpetological collections of the Biozentrum Grindel und Zoologisches Museum (ZMH), Universität Hamburg (ZMH A10525, A10527, A10529), of the Naturhistorisches Museum (NMBE) Bern (NMBE 1060403: adult male, 71.2 mm SVL, length of left testis 8.5 mm), and of the Zoologisches Forschungsmuseum Alexander Koenig (ZFMK), Bonn (ZFMK 92810: adult female, 125.5 mm SVL, maximum oocyte diameter 1.0 mm). The adults were morphologically identified by characters given in Inger (1966), Manthey and Grossmann (1997), and Malkmus et al. (2002).

For molecular assignment of our specimens to populations with confirmed locality data a molecular barcoding approach was applied based on a 800 bp piece of the 16S rDNA (foreward: 16SC 5' GTRGGCCTAAAAGCAGCCAC - 3', 16SA-L CGCCTGTTTATCAAAAACAT, 16SCH TCAAHTAAGGCACAGCTTA, reverse: 16SD 5' - CTCCGGTCTGAACTCAGATCACGTAG - 3', 16SB-H CCGGTCTGAACTCAGATCACGT, Vences et al. 2005; Rafe Brown, pers. comm.). Total genomic DNA was extracted from macerated muscle tissue with peqGold Tissue DNA Mini Kits (PEQLAB Biotechnologie GmbH) or DNeasy® Blood & Tissue Kit (Qiagen) according to the manufacturer's protocols. Cycling conditions for amplification have been published previously by Hertwig et al. (2011). Sequencing was done in both directions by Microsynth AG (Balgach, Switzerland) and Macrogen Inc. (Seoul, Korea). Sequence editing and management was done with BioEdit 7.0.5.2 (Hall, 1999, www.mbio.ncsu.edu/BioEdit/), Chromas Lite 2.01 (Technelysium Pty. Ltd., www.technelysium.com), and Geneious Pro 5.1.7 (Drummond et al., 2009) software.

The sequences were compared with samples of different populations of *M. nasuta* from the sequence database of the frogsofborneo.org project. Alignment was performed with MAFFT (Katoh et al. 2002) using the plugin of Geneious Pro with the E-INS-i algorithm and standard parameters. Genetic distances were obtained and visualized with the Geneious Pro tree builder with a neighbor-joining algorithm and the Tamura-Nei model of sequence evolution. The specimens from the breeding project were closely related to *M. nasuta* from Borneo. The lowest genetic distances of 1.2 and 1.4% respectively were found for two samples from a lowland population of this species inhabiting the Gunung Mulu National Park, Sarawak, Malaysia. This result is interpreted as indication of a possible origin of the founder animals of our breeding group from Borneo.

We photographed larval stages by placing single larvae into water filled glass vessels. Some photographs were used for ink drawings. A few freshly dead larvae at different developmental stages (Gosner stages 21, 25, 34, 39, and 44) that were first fixed in 4% formalin for some hours and subsequently preserved in 70% ethanol were used for morphological examination of character states with a Leica binocular microscope. These larvae were subsequently deposited in the collections of the Naturhistorisches Museum Bern (NMBE 1060404 [3 tadpoles]: stage 21, from 2010; stage 25, from January 2010; stage 44, from December 2009), and of the Zoologisches Forschungsmuseum Alexander Koenig, Bonn (ZFMK 92811: stage 34, from January 2010; ZFMK 92812: stage 39, from January 2010; ZFMK 92813, 92814: stage 44, from December 2009).

Abbreviations are as follows: GH – total hardness, KH – carbonate hardness; n = number; pH – pH value; TL = total length; terminology of larval morphology followed Altig and McDiarmid (1999) and Grosjean (2005).

Captive management of adults

Megophrys nasuta were maintained at the Amphibian Breeding Unit at Cologne Zoo without public access. Adults were housed in terrariums (L145 × W60 × H56 cm) that were divided into an aquatic and terrestrial section (Fig. 1a). The back and side walls of the terrariums were covered with artificial rock like decorative substrate. The terrestrial substrate consisted of a 20 cm thick layer of leaf litter covered with about five cm of dry leaves. Measurements of the surface of the aquatic section were L72.5 × W60 cm and water depth was about 10 cm with a total volume of 40 L. The water was connected to an external filter (EHEIM professional, Type 2224) with a capacity of 700 L/h.

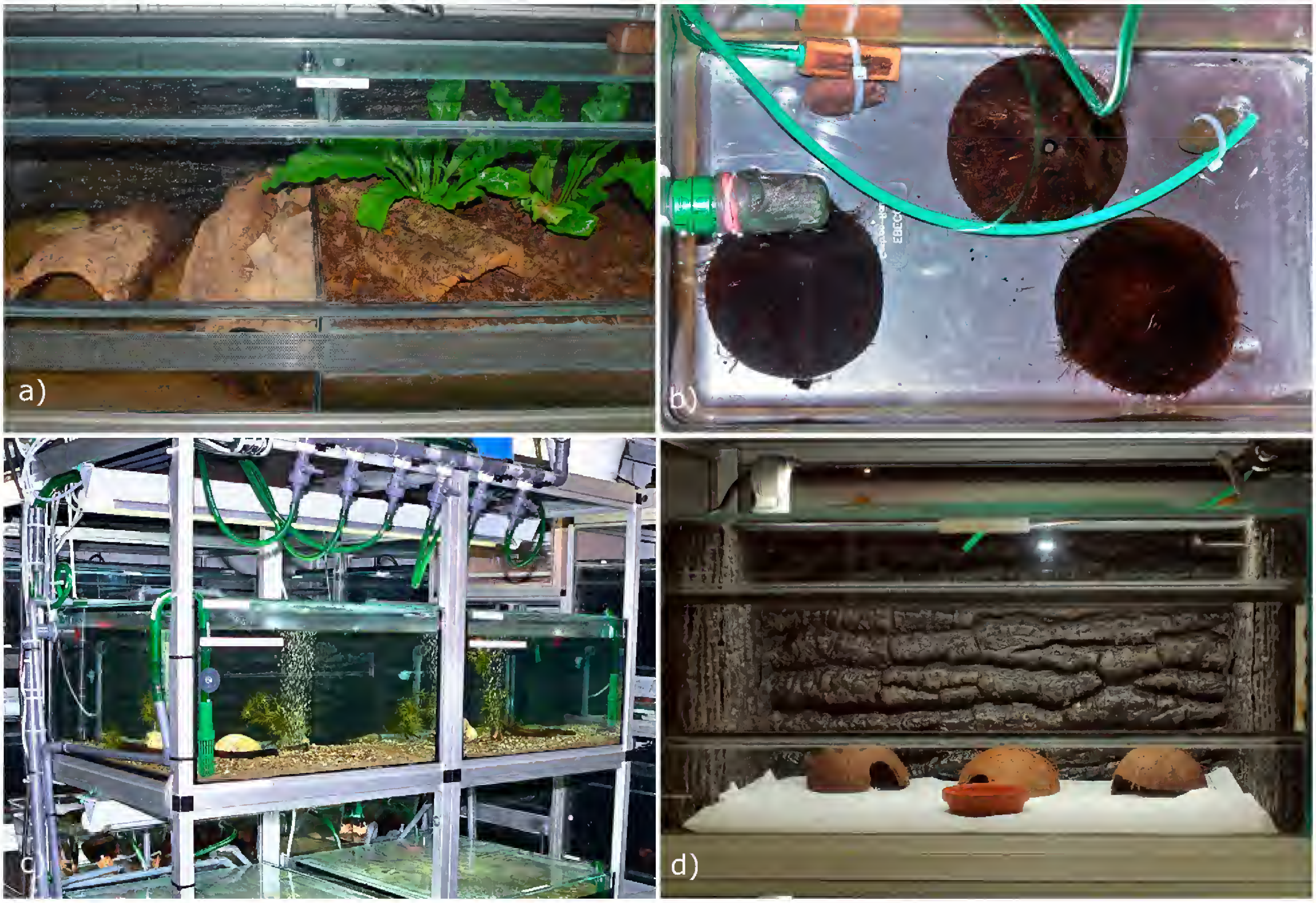


Figure 1. *Megophrys nasuta* enclosures in the amphibian breeding unit at the Cologne Zoo: a) terrarium of the adults, b) rearing tank for larvae at early developmental stages, c) aquaria for advanced larval stages, and d) rearing terraria for juveniles. *Photos: D. Karbe.*

In order to provide ready accessibility from the aquatic to the terrestrial section, as well as to provide oviposition sites, half of a cork tube was placed in the water. The terrestrial section included plants (*Asplenium nidus*) and cork tubes for shelter. Illumination was provided by fluorescent tubes (Namiba compact lights, UV replux: 36 Watt) and timer maintained photoperiod between 10 and 12 hours. Average temperatures were kept at 24-25 °C, and the humidity 80-100% through the use of a manual pump sprayer.

Captive management of larvae

Eggs were left in terrarium until hatching. The rearing tanks for larvae at early stages consisted of plastic tanks containing 13 L of water which were attached to an external filtration system (Eheim). After the hatching of the tadpoles more halves of coconut shells or cork pieces, and floating plants were added to provide hiding places (Fig. 1b). To ensure a constant water quality, part water changes were conducted every second day. Two months after hatch the tadpoles were transferred into aquariums (L54 × W65 × H30 cm), containing approximately 90 L of water, with a sand substrate and floating plants (Fig. 1c). Aquaria were connected to external filters with a 77 L filter volume which were run through 7 L pumps (Eheim).

Partial water changes were continued every second day; in addition, Catfish (*Corydoras*) were introduced to minimize the water contamination through uneaten feed. Lighting was provided by T5 fluorescent tubes (Osram FQ, 865 Lumilux daylight: 54 Watt), and water parameters were: temperature 24-27 °C (unless otherwise noted, see Table 1), pH 8.3, conductivity 320 µS, KH 2-4, and GH 6-8. Shortly before tadpoles metamorphosed, water level was reduced from 25 to 15 cm and a terrestrial section of 54 × 10 cm was established.

Captive management of metamorphs and juveniles

Metamorphs and juveniles were kept in groups of 20-30 specimens in terrariums measuring L60 × W45 × H30 cm that included a small water basin (maximum depth eight mm) and coconut husks for hiding places (Fig. 1d). For hygienic reasons, the substrate was paper tissue. Because the temperature should not exceed 23 °C, no additional illumination was used. To maintain a high humidity level, the terrarium was sprayed daily and front panels were tightly shut. Juveniles were reared to 2-4 cm and then transferred to other interested European institutions.

Nutrition

Adults were fed two or three times a week during their active periods, mostly on different invertebrates (house crickets, locusts, cockroaches), and infrequently (two times per month) on earthworms and newborn mice. Froglets were fed fruit flies (*Drosophila*) and then small house crickets (*Acheta domestica*) each day. All insects were fed a high quality herbal nutrition and dusted with minerals and vitamins (Korvimin ZVT + Reptil/Calcamineral). Tadpoles were fed on fine ornamental fish food (TetraMin). Feeding was introduced carefully when the first larvae were observed swimming at the water surface. When all tadpoles fed, food was applied 6-8 times a day, and later in the developmental progress feeding times were reduced to 2-4 times a day.

Results

Reproduction and larval development

Breeding was stimulated by providing a drier phase to the habitat, with reduced water level, during which terrarium was sprayed only as necessary for required humidity. This treatment was then followed by an artificial rain period, with rising water level and strong daily spraying, in order to simulate a natural rainy period. After beginning the artificial rain period, males that were discernible by their smaller size, darker throats and distinct nuptial pads, started calling (Fig. 2a). The loud, metallic calls first occurred at night, but with further breeding stimulation the males also began calling during the day.

Periods of calling were interspersed with inguinal amplexus, sometimes lasting several weeks, but did not necessarily lead to oviposition. Ovipositions were not seasonal, and were observed during January, May, June, July, October, and November (Fig. 2b). The minimum interval between ovipositions was about a month, but as several females housed with the males, we could not be sure of which females spawned. During night, eggs were deposited in clutches under the cork tube in water.

The white eggs were glutinous, attached to each other, and measured about two mm in diameter (Fig. 2b). Larvae hatched about one week after egg deposition with the yolk reservoir clearly visible (Figs. 2c, 2d). Between 50 and 300 larvae hatched per oviposition. Immediately after hatching, the larvae preferred dark hiding places such as under cork pieces or halved coconut shells. About ten days after hatching, the larvae developed a brownish pigmentation; at this stage the tadpoles remained clustered in close groups on the bottom.

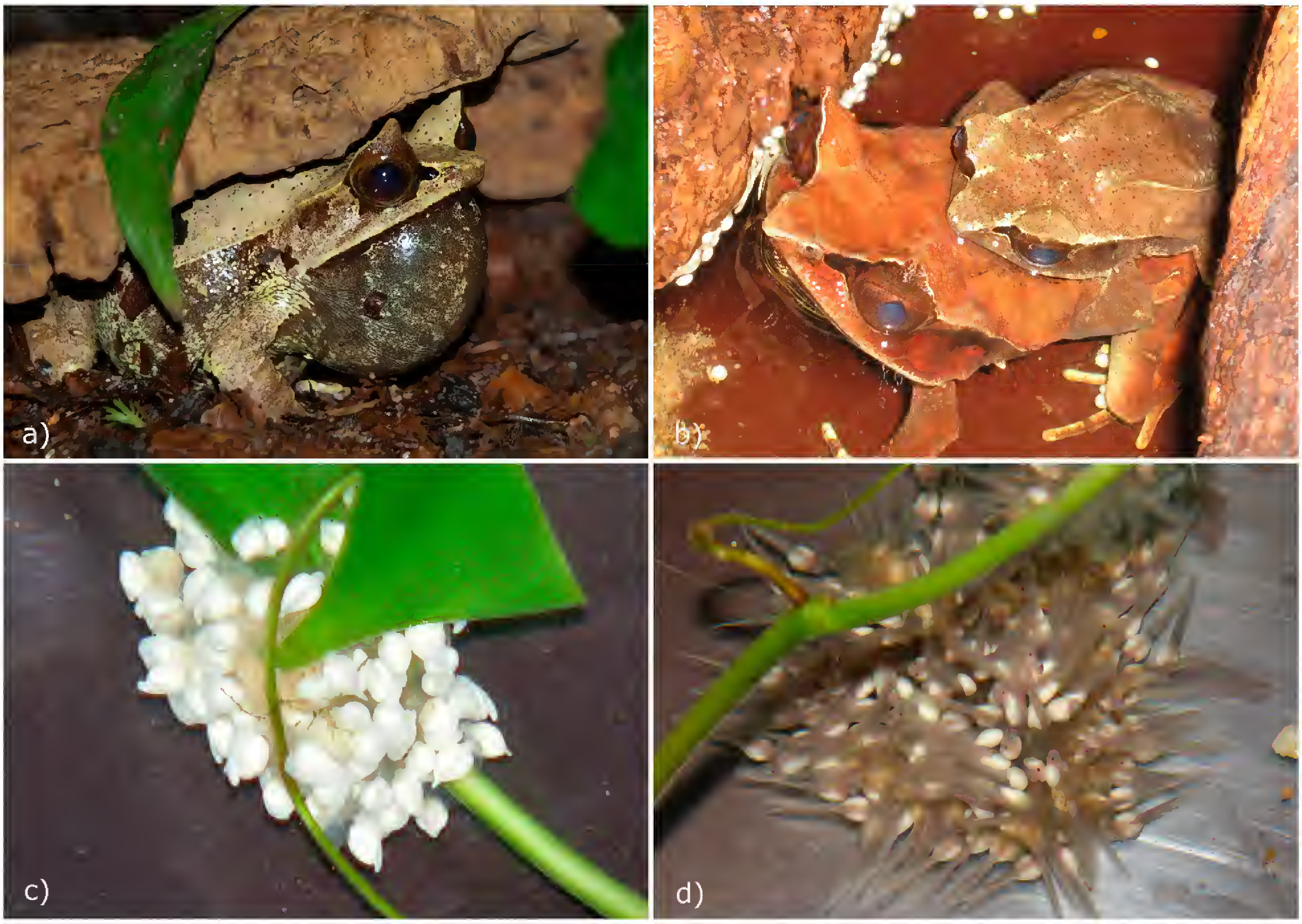


Figure 2. *Megophrys nasuta* at the amphibian breeding unit at the Cologne Zoo a) calling male, b) couple in amplexus during egg deposition, c) embryos, and d) hatched larvae with yolk sacs. *Photos: D. Karbe, A. Heidrich, T. Ziegler.*

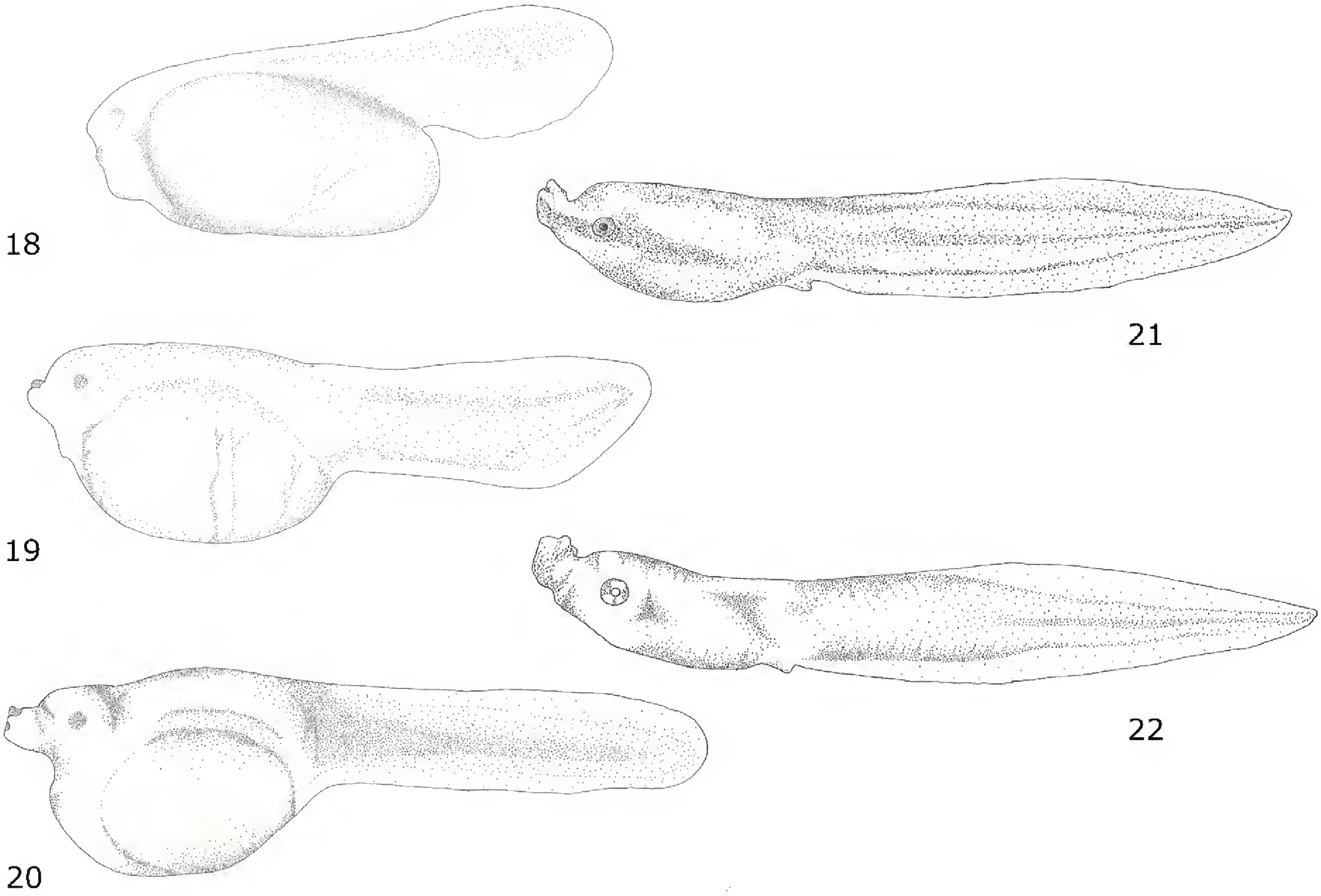


Figure 3. *Megophrys nasuta* larvae in stages 18 to 22. *Drawings: R. Bach.*

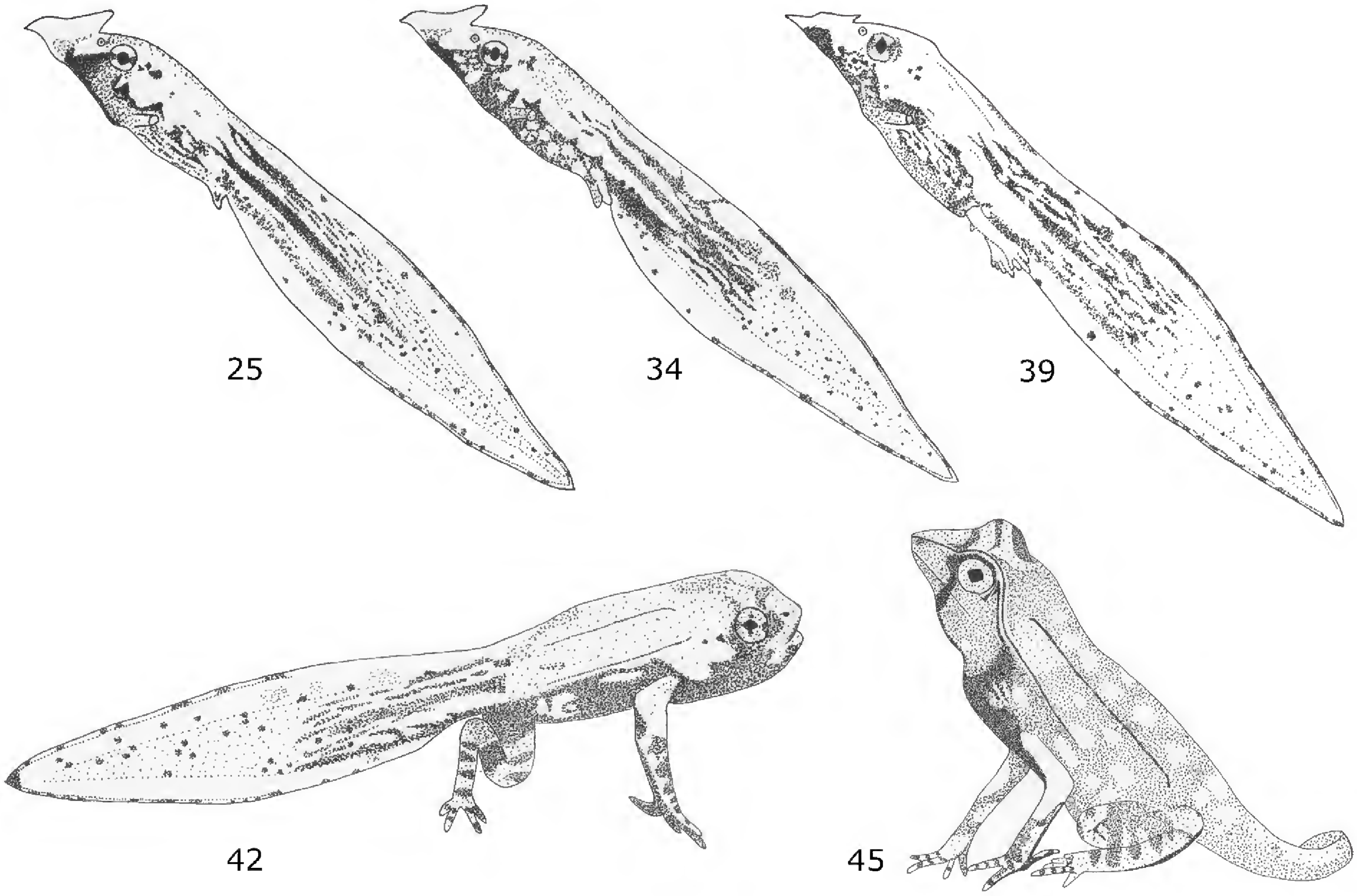


Figure 4. *Megophrys nasuta* larvae in stages 25 to 45. *Drawings: M. Wildenhues.*

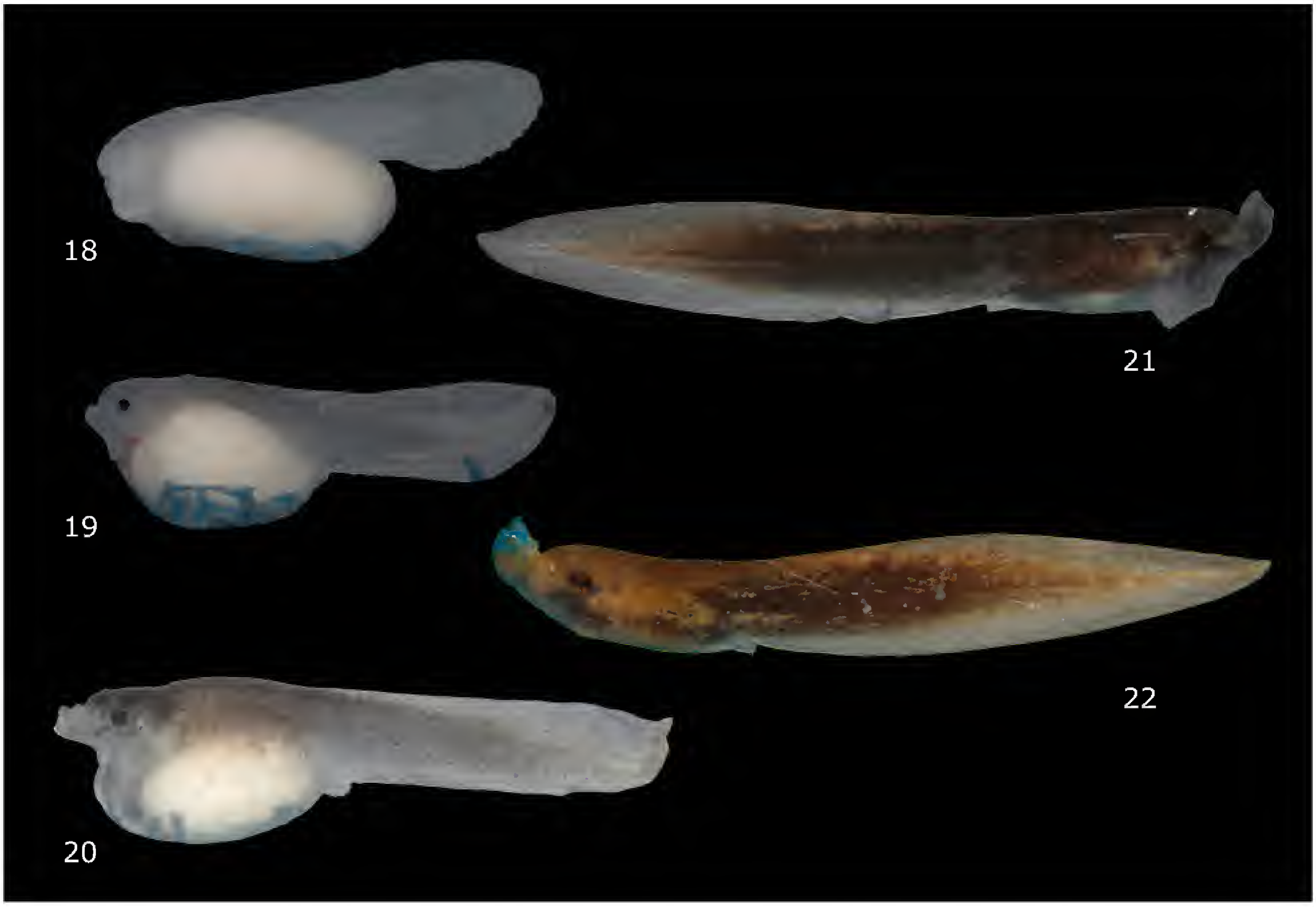


Figure 5. *Megophrys nasuta* larvae in stages 18 to 22; blue color is caused by the blue cellular material at the aquarium ground / background while taking photographs. *Photos: R. Bach, T. Ziegler, D. Karbe.*

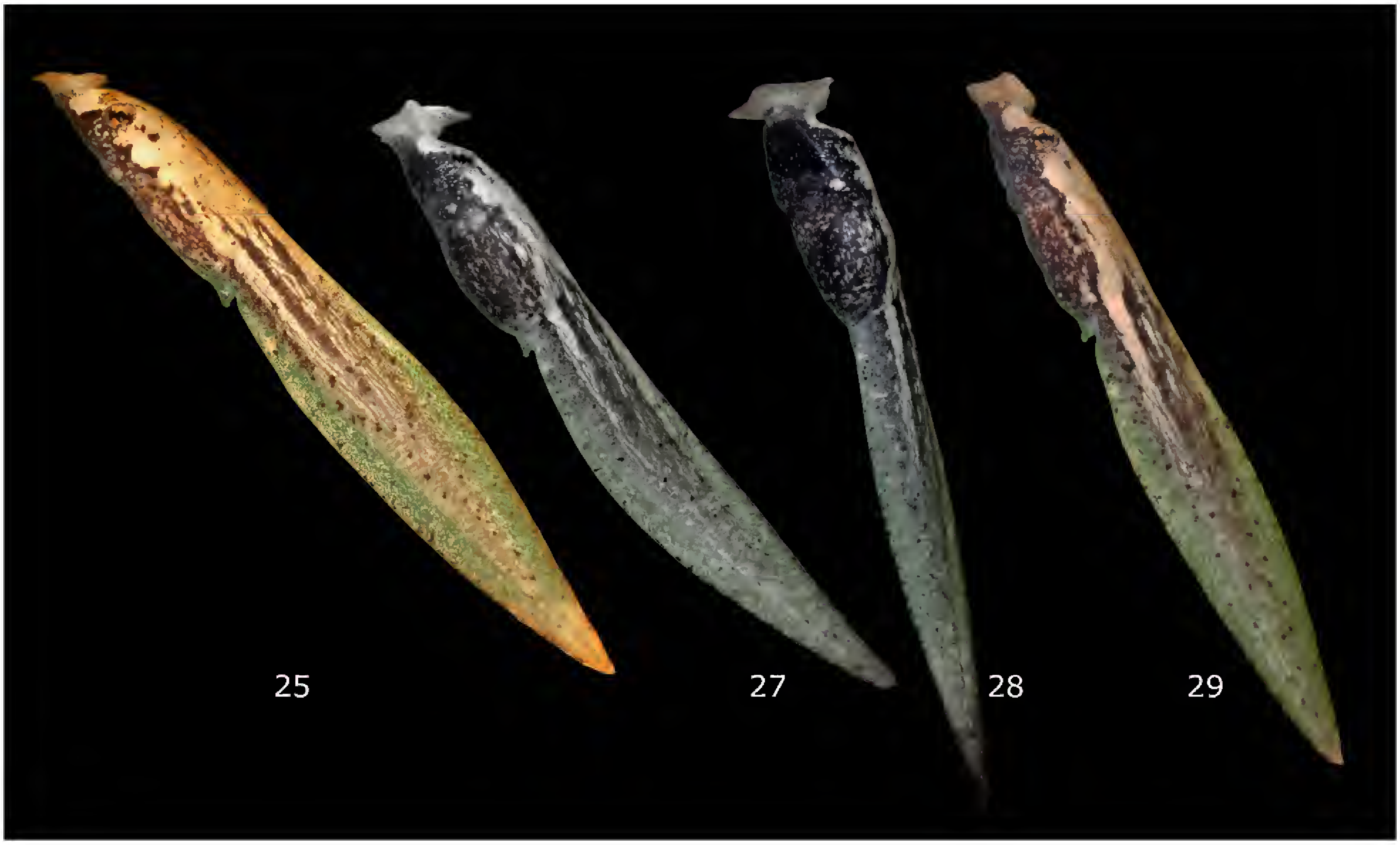


Figure 6. *Megophrys nasuta* larvae in stages 25 to 29. *Photos: M. Wildenhues.*

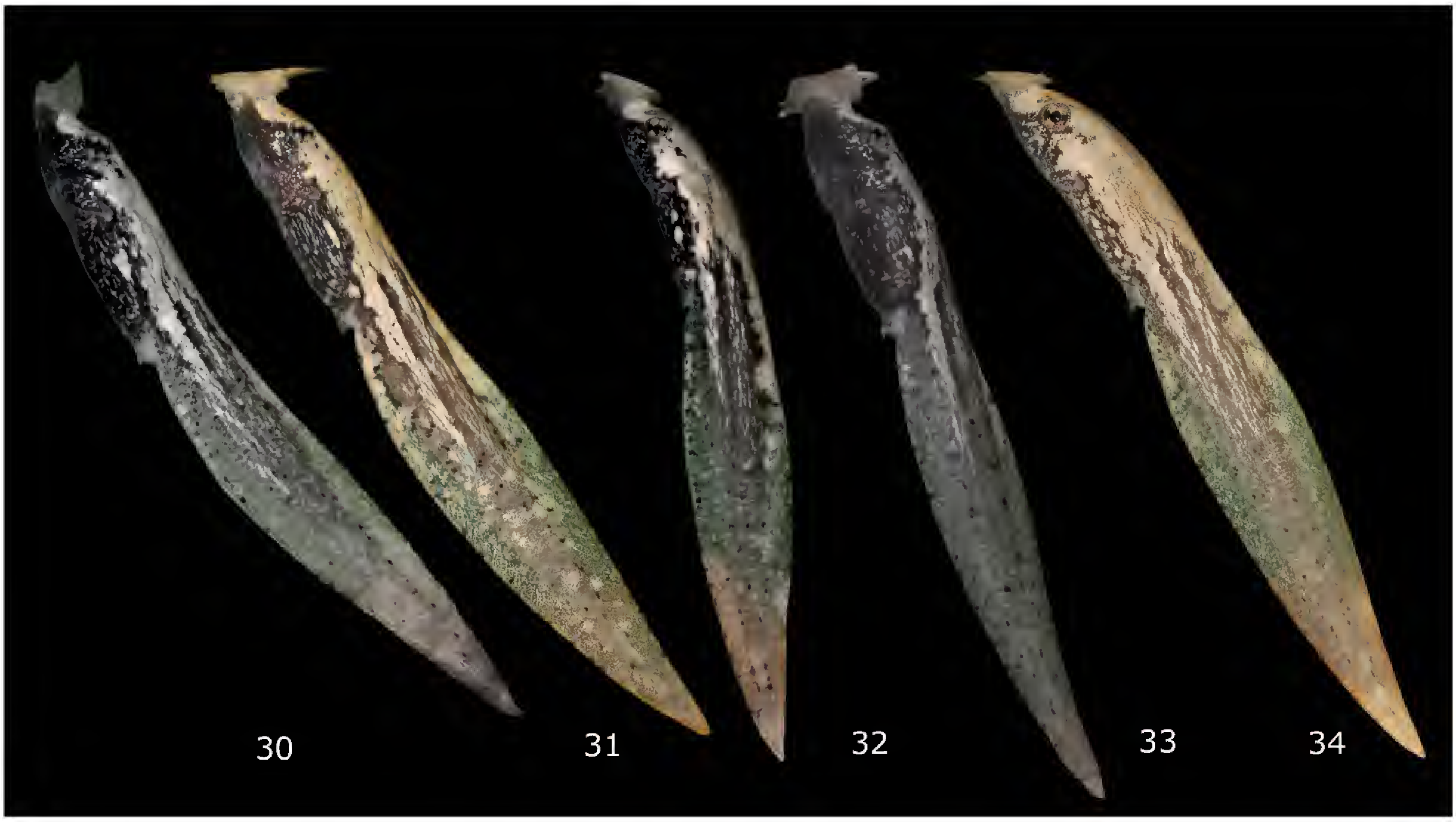


Figure 7. *Megophrys nasuta* larvae in stages 30 to 34. *Photos: M. Wildenhues.*

For detailed staging of the following early developmental stages see Table 1. The funnel mouth became discernible about one week after hatch. About four days later, the larvae began to move to the water surface, and after about two weeks after hatch all tadpoles were feeding. Three weeks after hatch the tadpoles had reached lengths of up to two cm. For detailed staging of the following advanced developmental stages see Table 2. After about nine weeks after hatch, some tadpoles showed a distinct ventral pattern. On average around sixty days after hatch, at Gosner stage 26 or 27, hind limbs started to develop. At this time, the largest tadpoles measured about 4.5 cm, and feeding times were reduced to two times a day because of their good nutritional condition. Shortly before metamorphosis the funnel mouth was reduced and dorsal coloration darkened.

About 2.5 months after egg deposition the first larvae moved onto the terrestrial section to metamorphose. At that time the metamorphs had body lengths of 15-18 mm. Reabsorption of the tail took two or three days, the triangular projections at the upper eyelids, which are characteristic for the advanced terrestrial stages, began to develop after about two or three weeks after completion of metamorphosis. While most of the larvae had finished their development and commenced with metamorphosis after 3.0-3.5 months, some individuals showed a distinctly slower developmental progress which took up to seven months, or longer in some cases. Larval development was both temperature and density dependent.

We generally observed a faster growth at higher water temperatures. For example, larvae that were kept at minimum temperatures of 24 °C developed dark pigmentation ten days after hatch, whereas larvae kept at minimum temperatures of 22 °C developed dark pigmentation up to six days later (see Table 1). Another example from early development is the formation of the funnel mouth, which can occur 2-3 weeks after egg deposition dependent on different temperature conditions (see also Table 1). In addition, larvae kept in smaller groups (ca. 10-15 per rearing tank) grew faster compared to similar larvae in tanks with a higher density.

Morphology of developmental stages

We documented the larval development in *Megophrys nasuta* using Gosner (1960) larval stages, as reproduced in Altig and McDiarmid (1999), to describe diagnostic larval characters and stages. For developmental stages 18-22 we assessed diagnostic morphological features and age in days based on 2-6 individuals (see Table 1 and Figs. 3 and 5). For morphological description of developmental stages 25-46 (see Table 2 and Figs. 4, 6-9), we increased the number of larvae up to 12 individuals and measured length instead of age in days.

Compared to standard developmental tables, proposed for most other anuran species (e.g., Pan and Liang 1990), the funnel-shaped oral disc of tadpoles, typical for other megophryid genera (such as *Brachytarsophrys*, or *Xenophrys*), served as an additional character for staging. We have not presented a detailed morphological larval description in an advanced stage because several papers have already described these. General larval views including short descriptions were provided (e.g.,

Table 1. Developmental stages of *Megophrys nasuta* bred at the Cologne Zoo from stage 18-22, including age and diagnostic features (n = 2-8). Some of the larvae were reared under lower water temperatures than previously described (minimum value ca. 22 °C) which explains the somewhat slower development compared with tadpole growth described in results; stage diagnostic characters according to Gosner (1960) are in italics. [1]Could not be observed in our sample.

Stage number	Age (days)	Diagnostic features
18	11 (n = 2)	*muscular response to water movement*; eye region begins to develop
19	16 (n = 8)	*heart beat visible*; eye pigmentation distinctly discernible; oral region begins to stretch upwards; developing dark pigmentation on body dorsum and tail; *yolk reservoir reduced and blood vessels discernible*
20	- (n = 5)	(*development and circulation of external gills*[1]); elongated oral region; last stage with distinctly visible yolk reservoir; tail longer than body
21	~21 (n = 7)	*cornea transparent*; funnel mouth discernible; dark body and tail musculature with transparent and distinctly developed fin
22	60 (n = 7)	*fin circulation begins*; dark dorsal pigmentation brightens

by Nodzenski et al. 1989, including the description of the visceral organization; Manthey and Grossmann 1997; and Malkmus et al. 2002); more detailed larval drawings (including lateral and oral disc) were provided by Schmidt (1976). The most detailed descriptions are in Inger (1966: under the name *M. monticola nasuta*), Inger (1985), who described internal buccopharyngeal morphology including scanning electron microscopy, and Leong and Chou (1999) (see also Das and Haas 2005).

Discussion

During keeping and breeding of *Megophrys nasuta* at Cologne Zoo we found drier conditions followed by phases of intense water spraying (rain simulation) to be important triggers for subsequent reproductive behavior and reproduction. Similar observations have been made by other authors (see Table 3b). In contrast to Pfeuffer (1989), who only noticed mating during increased temperatures in spring, we did not recognize seasonal related breeding behavior. Pfeuffer (1989) also observed egg depositions only during the daytime, whereas ovipositions at Cologne Zoo only took place during dusk and night (see also Schmidt 1976, 1977, Table 3b). In addition, we realized that housing several males with females stimulated mating, probably because of male-male competition.

We observed a wide variation in developmental time of *M. nasuta*. Whereas the first tadpole finished metamorphosis about 2.5 months after egg deposition, others did not metamorphose for seven months. We cannot know whether this wide variation also takes place under natural conditions or whether this is due to the artificial environment. Dependent on the species and the rearing conditions captive bred individuals, even in the first generation, may not be physiologically equivalent to wild individuals (Ron Altig, in litt.).

Nevertheless, mean developmental times at water temperatures of 24-26 °C were 2.5-3.5 months. We reared *M. nasuta* larvae under different water temperatures and observed development was faster at higher water temperatures. Schmidt (1977) also observed faster growth at higher temperatures of larvae kept at 22-28 °C compared with larvae reared at 19-20 °C. Development under natural conditions may also take longer than in our study because water temperatures of 18-21 °C were found in the habitat of *M. nasuta* (Malkmus 1995).

Lower density of larvae in the rearing tanks also appeared to increase developmental rate (see also Schmidt 1976, 1977) perhaps because of better accommodation and optimum nutrient availability in smaller groups. Thus, differences in temperature, population density, and greater nutrient supply appear to be the causes of different body sizes and development stages of tadpoles, of the same age. In general, larvae that developed faster led to comparatively smaller metamorphs and juveniles (e.g., 10 mm after 2.5 months developmental time versus 15-17 mm after 3.5 months). The effects of possible differences in metabolism or a different genetic background on development rates cannot be excluded. Further studies regarding the rearing of *M. nasuta* tadpoles might help to better understand factors that influence their development.

Appropriate staging of the larval period is fundamental to various life history studies of amphibians (e.g., Shimizu and Ota 2003). While trying to morphologically describe the larval stages of *M. nasuta*, we found differences compared with methodology applied by Gosner (1960). While Gosner stages 26-34 are characterized by development of hind limbs, such approach is difficult in *M. nasuta* because hind limbs of larvae are white during early development (as is likewise the case in other anurans). Although differentiation of these stages is possible to diagnose in life with a microscope or a hand loupe,

Table 2. Developmental stages of *Megophrys nasuta* bred at the Cologne Zoo from stage 25-46 including total lengths (TL) and diagnostic features (n = 1-12); stage diagnostic characters according to Gosner (1960) are in italics.

Stage number	TL (in mm)	Diagnostic features
25	22.07-30.94 (n = 6)	*spiracle opening sinistral*; pigmentation complete; funnel mouth complete
27	24.90-31.75 (n = 10)	hind limb buds visible; *length of hind limbs > 0.5 × basal width*
28	27.54-32.08 (n = 12)	*length of hind limbs > basal width*; length of hind limbs < length of vent tube
29	28.31-31.30 (n = 7)	*length of hind limbs > 1.5 × basal width*
30	30.78-34.85 (n = 8)	*length of hind limbs = 2 × basal width*; length of hind limbs = length of vent tube
31	33.35-34.85 (n = 2)	*foot paddle-shaped*
32	32.11 (n = 1)	*indentation between 4th and 5th toe*
33	30.37-34.08 (n = 3)	*indentation between 3rd and 4th toe*
34	31.39-34.10 (n = 5)	*indentation between 2nd and 3rd toe*
35	33.33-35.00 (n = 3)	*indentation of all toes*; hind limb > vent tube
36	33.30-36.54 (n = 4)	*toes 3-5 separated*
37	34.78-37.93 (n = 2)	*all toes separated*; pigmentation of hind limbs darkens
38	33.51-35.80 (n = 6)	*metatarsal tubercle visible*
39	32.56-35.62 (n = 2)	*subarticular patches slightly visible*
40	33.37-35.70 (n = 2)	fore limb bumps visible; hind limbs with distinct pattern; *last stage with vent tube*
41	31.63-32.40 (n = 2)	funnel mouth atrophy; *vent tube gone*
42	29.80-34.90 (n = 3)	funnel mouth degenerated; *fore limbs emerged*; spiracle opening disappeared; mouth beneath nostril
43	31.04 (n = 1)	snout pointed; eyeballs starting to protrude; *mouth between nostril and eye*
44	24.05-35.73 (n = 3)	terrestrial life modus; tail atrophy; eyeballs further pointed; longitudinal ridges on back; *mouth beneath eye*
45	15.50-18.20 (n = 3)	tail mostly reduced; *mouth posterior to eye*
46	–	change of pigmentation (cream, fawn); lappet of snout and eyeballs visible; ridges on back and head become more distinct; *tail completely resorbed*

such attempt is difficult based only on photographs. This is the reason why we could not provide photographic evidence at stage 26.

In contrast, the development of the funnel mouth and length of the hind limb bud compared to the vent tube serve as additional characters in early larval stages of *M. nasuta*. The atrophy of the funnel mouth, the eye development, and the longitudinal ridges serve as diagnostic features of the species' advanced stages. Compared with Gosner (1960), we could also observe that the development of the forelimb bumps and of mouth shape in relation to position of the nostril and eye developed formerly in *M. nasuta*. Further studies on the egg development of *M. nasuta* and descriptions of stages 23, 24, and 26 are required to complete our preliminary development table.

Outlook

In general, the megophryid *M. nasuta* is relatively easy to keep, presupposed that sufficient land and water space, appropriate climatic conditions, and sufficient substrate and hiding places are provided. Breeding is possible, when drier phases followed by subsequent intensive spraying, as important triggers for reproductive activities, are provided. During the rearing of larvae, tanks must be clean, group sizes should not be too large, and a continuous, multiple feeding per day (in particular) during early larval development should be provided. In addition, sufficient filtration and proper water exchange must be guaranteed. The rearing of the metamorphs and juveniles is time consuming but feasible.

M. nasuta is a large and attractive anuran with interesting ecological adaptations such as camouflage and somatolysis (figure dissolution) and thus is quite suitable for public zoo exhibits. This species occurs in high numbers in the international pet trade, and while few captive breeding successes have been reported, we would like to encourage other zoos and amphibian keeping facilities to keep and breed this species. Breeding activities under captive conditions, such as in zoos, especially with focus on amphibians, might considerably help to reduce the number of wild caught *M. nasuta* by providing this demand with captive bred individuals.

However, there are less understood and more endangered megophryids than *M. nasuta*, such as some of the *Megophrys* congeners, for which this overview paper might be a useful guide in future conservation breeding programs. For such conservation breeding purposes, the parental generation should at least have proper locality information or should be genetically screened, because there is still some taxonomic uncertainty among

Table 3a. Basic husbandry parameters based on the papers by Schmidt (1976, 1977), and Pfeuffer (1989) in comparison with our own results.

	Schmidt (1976, 1977)	Pfeuffer (1989)	Wildenhues et al. (2012)
adult husbandry			
terrarium size (cm)	120 × 70 × 100	85 × 60 × 50	145 × 60 × 56
land (cm)	30 × 50 (foam material)	42.5 × 60 (foam & synthetic rubber)	72.5 × 60 (leaf litter)
water depth (cm)	8	8	10
equipment	cork tubes, *Scindapsus*, *Philodendron*	cork tube caves, roots, flat stones, twine	cork tubes, *Asplenium nidus*
illumination	–	fluorescent tubes (20 Watt)	fluorescent tubes (54 Watt)
temperature	not exceeding 25 °C (preferred temperature up to 22 °C)	ca. 22-25 °C	24-25 °C
heating	–	slight floor heating	–
nutrition	crickets, earthworms, newborn mice	everything they could swallow	crickets, earthworms, newborn mice
larval husbandry			
water parameters	temperature 24 °C, GH 12.5, KH 9.5, pH 7.8	temperature 24-26 °C	temperature 24-27 °C, GH 6-8, KH 2-4, pH 8.3, conductivity 320 µS
juvenile husbandry			
terrarium size (cm)	100 × 40 × 30 (*n* = 102 froglets)	–	60 × 45 × 30 (*n* = 20-30 froglets)
	19 × 19 × 8.5 (*n* = 12 froglets)	–	–
equipment	synthetic foam, cork pieces	–	paper tissues, coconut husks
nutrition	small crickets, house crickets, wax and flour moth larvae	small earthworms, slugs	fruit flies, later on small house crickets

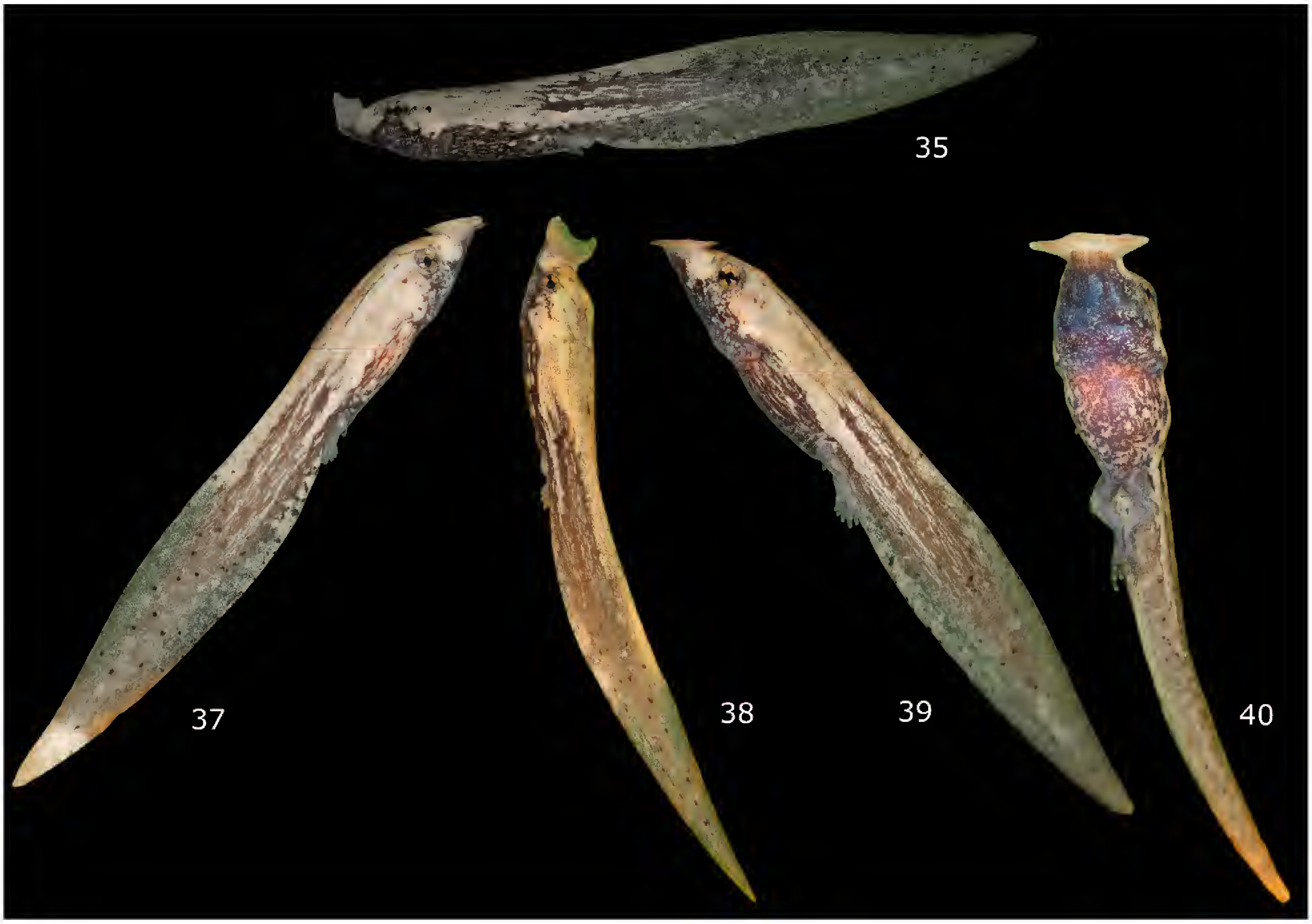


Figure 8. *Megophrys nasuta* larvae in stages 35 to 40. *Photos: M. Wildenhues.*

Table 3b. Breeding data based on the papers by Schmidt (1976, 1977), Pfeuffer (1989), and Anonymous (1994) compared with our own results; [1]when eggs were removed from the water part of the terrarium and fungus was eliminated; [2]when eggs remained in the water part of the terrarium; [3]before the development of the funnel mouth, larvae proved to be sensitive towards low temperatures (fatalities occurred at 18-20 °C); [4]after egg deposition.

	Schmidt (1976, 1977)	**Pfeuffer (1989)**	**Anonymous (1994)**	**Wildenhues et al. (2012)**
calls	from middle of December onwards, at dusk	throughout the whole year, most common during spring, at that time also during daytime	–	after beginning of rain period, first at night, later also during daytime
oviposition months, and time	December, July and August, at night	March, 10:00-18:00	August, during artificial rain period	January, May, June, July, October, and November, at night
egg number	1,474-2,033	1,500-2,000	~ 300	–
hatching[4]	6 days	~ 4 days	one week	~ one week
hatching success	6-26%[1] or 72-88%[2]	~ 90%	–	–
first feeding[4]	~ 25 days	–	–	~ 20 days
developmental time (from egg deposition onwards)	first metamorphosis took place after 3 months[3]	first metamorphosis took place after 4 months	–	first metamorphosis took place after 2.5 months
froglet size after metamorphosis (cm)	1.0-1.6	1 or 2	–	1.0-1.7

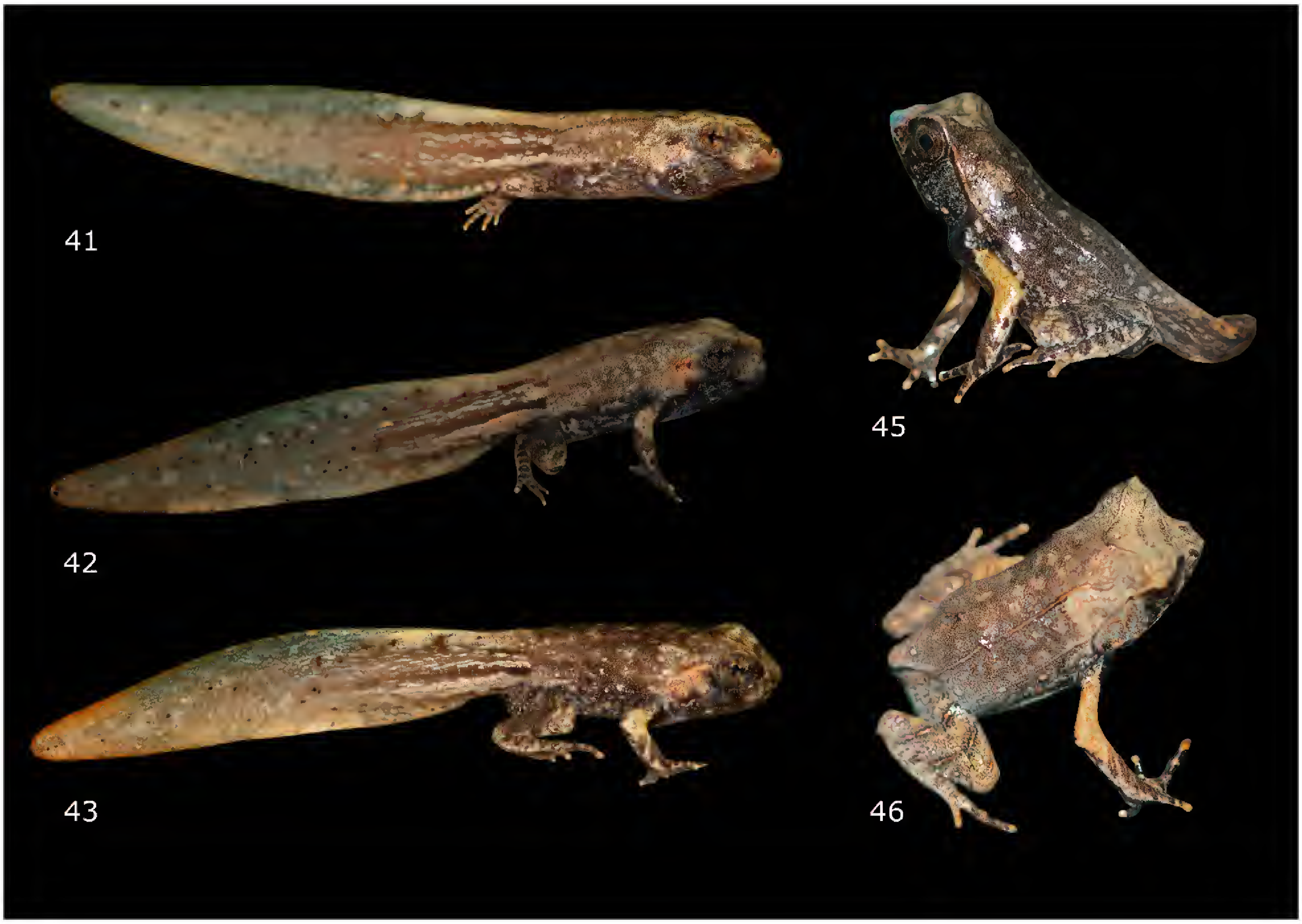


Figure 9. *Megophrys nasuta* larvae in stages 41 to 46. *Photos: M. Wildenhues.*

megophryids and species descriptions pending. A good example is the only recently described, endemic *M. kobayashii*, IUCN status near threatened and is only known from a geographically very limited range (Borneo's Mount Kinabalu, the Crocker Range, and Mount Trus Madi, in Sabah, Malaysia, at 1,300-1,600 m elevation; Frost 2011).

Acknowledgments.—The senior author would like to thank Anna Gawor (Cologne) for her kind support. Many thanks also to Professor Dr. Alexander Haas (University Hamburg) for initiating molecular comparisons with specimens collected by his working group. Professor Dr. Ronald Altig (Mississippi State University), Dr. Robert Browne (Sartenaja, Belize), and an anonymous reviewer kindly helped to improve a previous version of the manuscript. Thanks also to Lieselotte Schulz (Cologne Zoo) for her support in record keeping and to Astrid Heidrich, Perth, for providing a photograph taken at Cologne Zoo.

Literature cited

ALTIG R, MCDIARMID RW. 1999. *Tadpoles: The biology of anuran larvae*. The University of Chicago Press, Chicago, London.

ANONYMUS 1994. Erfolgreiche Nachzucht des Zipfelfrosches. *DATZ* 47(6):346.

BERGER L, SPEARE R, DASZAK P, GREEN DE, CUNNINGHAM AA, GOGGIN CL, SLOCOMBE R, RAGAN MA, HYATT AD, MCDONALD KR, HINES HB, LIPS KR, MARANTELLI G, PARKES H. 1998. Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. *Proceedings of the National Academy of Sciences of the United States of America* 95(15):9031-9036.

BRIGGS CJ, VREDENBURG VT, KNAPP RA, RACHOWICZ LJ. 2005. Investigating the population-level effects of chytridiomycosis: An emerging infectious disease of amphibians. *Ecology* 86(12):3149-3159.

BROWNE RK, WOLFRAM K, GARCIA G, BAGATUROV MF, PEREBOOM ZJJM. 2011. Zoo-based amphibian research and conservation breeding programs. *Amphibian and Reptile Conservation* 5(3):1-14.

DAS I, HAAS A. 2005. Sources of larval identities for amphibians from Borneo. *Herpetological Review* 36(4):375-382.

DRUMMOND AJ, ASHTON B, CHEUNG M, HELED J, KEARSE M, MOIR R, STONES-HAVAS S, THIERER T, WILSON A. 2009. Geneious v4.7. [Online]. Available: www.geneious.com [Accessed: 23 July 2009].

FROST DR. 2011. Amphibian Species of the World: An Online Reference. Version 5.5 (31 January 2011). [Online: Electronic Database. American Museum of Natural History, New York, New York]. Available: http://research.amnh.org/vz/herpetology/amphibia/ [Accessed: 18-01-2012].

GOSNER KL. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* 16:183-190.

GRIFFITHS RA, PAVAJEAU L. 2008. Captive breeding, reintroduction, and the conservation of amphibians. *Conservation Biology* 22(4):852-861.

GROSJEAN S. 2005. The choice of external morphological characters and developmental stages for tadpole-based anuran taxonomy: A case study in *Rana* (Sylvirana) nigrovittata (Blyth, 1855) (Amphibia, Anura, Ranidae). *Contributions to Zoology* 74(1/2):1-22.

HALL TA. 1999. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41:95-98.

HERTWIG ST, DAS I, SCHWEIZER M, BROWN R, HAAS A. 2011. Phylogenetic relationships of the *Rhacophorus* everetti-group and implications for the evolution of reproductive modes in *Philautus* (Amphibia: Anura: Rhacophoridae). *Zoologica Scripta* 41(1):9-46.

INGER RF. 1954. Systematics and Zoogeography of Philippine Amphibia. *Fieldiana Zoology* 33:183-531. Natural History Museum, Chicago, Illinois. 531 p.

INGER RF. 1966. *The Systematics and Zoogeography of the Amphibia of Borneo*. Fieldiana Zoology 52. Field Museum of Natural History, Chicago, Illinois. 402 p.

KATOH K, MISAWA K, KUMA K, MIYATA T. 2002. MAFFT: A novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research* 30(14):3059-3066.

LEONG TM, CHOU LM. 1999. Larval diversity and development in the Singapore Anura (Amphibia). *The Raffles Bulletin of Zoology* 47(1):81-137.

MAHONY S. 2011. Two new species of *Megophrys* Kuhl & van Hasselt (Amphibia: Megophryidae), from western Thailand and southern Cambodia. *Zootaxa* 2734:23-39.

MALKMUS R. 1995. Die Krötenfrösche der Gattung *Megophrys* KUHL & VAN HASSELT, 1822 am Mount Kinabalu, Nord - Borneo. *Herpetofauna* (Weinstadt) 17(94):6-10.

MALKMUS R, MANTHEY U, VOGEL G, HOFFMANN P, KOSUCH J. 2002. *Amphibians and Reptiles of Mount Kinabalu (North Borneo)*. Ruggell (A.R.G. Gantner Verlag K.G.). 424 p.

MANTHEY U, GROSSMANN W. 1997. *Amphibien und Reptilien Südostasiens*. Natur- und Tier Verlag, Münster. 512 p.

MCGREGOR REID G, ZIPPEL KC. 2008. Can zoos and aquariums ensure the survival of amphibians in the 21st century? *International Zoo Yearbook* 42:1-6.

MENDELSON JR III, LIPS KR, GAGLIARDO RW, RABB GB, COLLINS JP, DIFFENDORFER JE, DASZAK P, IBANEZ R, ZIPPEL KC, LAWSON DP, WRIGHT KM, STUART SN, GASCON SC, DE SILVA HR, BURROWES PA, JOGLAR RL, LA MARCA E, LÖTTERS S, DU PREEZ L, WELDON C, HYATT A, Rodrigues-Mahecha JV, Hunt S, Robertson H, Lock B, Raxworthy CJ, Frost DR, Lacy RC, Alford RA, Campbell JA, Parra-Olea G, Bolanos F, Domingo JJC, Halliday T, Murphy JB, Wake MH, Coloma LA, Kuzmin S, Price MS, Howell KM, Lau M, Pethiyagoda R, Boone M, Lannoo MJ, Blaustein AR, Dobson A, Griffiths RA, Crump ML, Wake DB, Brodie Jr ED. 2006. Biodiversity - Confronting amphibian declines and extinctions. *Science* 313(5783):48.

Nodzenski E, Wassersug RJ, Inger RF. 1989. Developmental differences in visceral morphology of megophryine pelobatid tadpoles in relation to their body form and mode of life. *Biological Journal of the Linnean Society* 38(4):369-388.

Pan J, Liang D. 1990. Studies of the early embryonic development of *Rana rugulosa* Wiegmann. *Asiatic Herpetological Research* 3:85-100.

Pfeuffer FJ. 1989. Der Zipfelfrosch *Megophrys monticola nasuta*. *DATZ* 42(3):150-151.

Rogner M. 1980. Viel Freude mit Zipfelkrötenfröschen, *Megophrys nasuta*. *Aquarium Aqua Terra* 14(134):425-427.

Schlegel H. 1858. *Handleiding tot de Beoefening der Dierkunde.* Volume 2. Breda: Koninklijke Militaire Akademie. 57 p.

Schmidt AA. 1976. Erst-Nachzucht des Zipfelfrosches *Megophrys nasuta* (Amphibia, Salientia, Pelobatidae). *Salamandra* 12(2):55-68.

Schmidt AA. 1977. Weitere Beobachtungen bei der Nachzucht des Zipfelfrosches (*Megophrys nasuta*). *Salamandra* 13(1):43.

Schmidt AA, Wicker R. 1977. Weitere Beobachtungen bei der Nachzucht des Zipfelkrötenfrosches *Megophrys nasuta*. *Salamandra* 13(1):43-48.

Schwanz S. 1979. Der Zipfelkrötenfrosch. *Aquarien* (Magazine) 13(12):610-612.

Shimizu S, Ota H. 2003. Normal development of *Microhyla ornata*: The first description of the complete embryonic and larval stages for the microhylid frogs (Amphibia: Anura). *Current Herpetology* 22(2):73-90.

v. d. Nieuwenhuizen A. 2001a. *Megophrys monticola nasuta* (deel 1). *Aquariumwereld* 54(11):254-259.

v. d. Nieuwenhuizen A. 2001b. *Megophrys monticola nasuta* (slot). *Aquariumwereld* 54(12):285-289.

Van der Straeten K, Karbe D, Olbort S, Ziegler T. 2007. Erste Nachzucht des Zipfelkrötenfrosches, *Megophrys nasuta*, im Aquarium des Kölner Zoos. *Terraria* Nr. 6 2(4):45-51.

van Dijk PP, Iskandar D, Inger R. 2004. *Megophrys nasuta*. In: IUCN 2011. IUCN Red List of Threatened Species. Version 2011.2. [Online]. Available: www.iucnredlist.org. [Accessed: 20 January 2012].

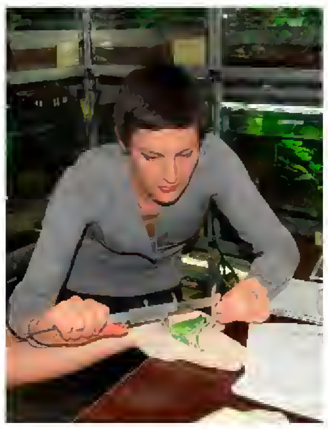
Vences M, Thomas M, Bonett RM, Vieites DR. 2005. Deciphering amphibian diversity through DNA barcoding: Chances and challenges. *Philosophical Transactions of the Royal Society B* 360:1859-1868.

Ziegler T, Dang TT, Nguyen TQ. 2011. Breeding, natural history and diversity research: *Ex situ* and *in situ* Asian amphibian projects of the Cologne Zoo and the Institute of Ecology and Biological Resources. In: *Biology and Conservation of Tropical Asian Amphibians*. Editors, I Das, A Haas, AA Tuen. Proceedings of the Conference "Biology of the amphibians in the Sunda region, South-east Asia," Sarawak, Malaysia, 28-30 Septemebr 2009 – Institute of Biodiversity and Environmental Conservation, Universiti Malaysia Sarawak, Kota Samarahan. 137-146.

Ziegler T, van der Straeten K, Karbe D. 2008. *Der Zipfelkrötenfrosch Megophrys nasuta*. Natur und Tier-Verlag, Münster. 63 p.

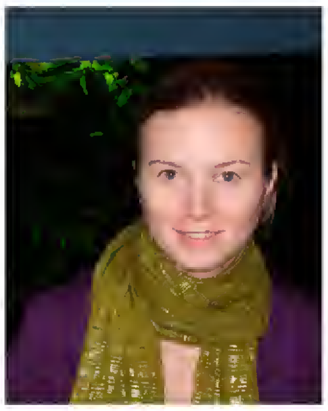
Zippel K, Johnson K, Gagliardo R, Gibson R, McFadden R, Browne R, Martinez C, Townsend E. 2011. The amphibian ark: A global community for *ex situ* conservation of amphibians. *Herpetological Conservation and Biology* 6(3):340-352.

Received: 03 February 2012
Accepted: 20 February 2012
Published: 24 March 2012

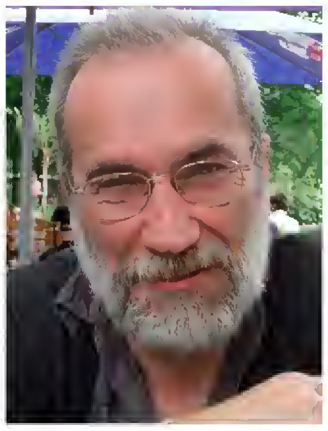


MARLEN WILDENHUES is working for the Rhine-Bergish District as a local species conservation appointee since 2011. Her interest for amphibians started with two practical courses on larval morphology and development at the Cologne Zoo in 2009, resulting so far in two scientific publications. She completed her Master Thesis in 2010 at the Faculty of Mathematics and Natural Sciences of the Rhineland Friedrich-Wilhelms-University Bonn in collaboration with the Cologne Zoo and the Institute of Ecology and Biological Resources in Hanoi Vietnam, focusing on the larval morphology and development of Vietnamese tree frogs.

ANNA RAUHAUS started her career at the Aquarium / Terrarium Department of the Cologne Zoo in May 2011. She finished her apprenticeship as zoo keeper in the year 2010. Her focus of expertise is herpetology and behavioral training.



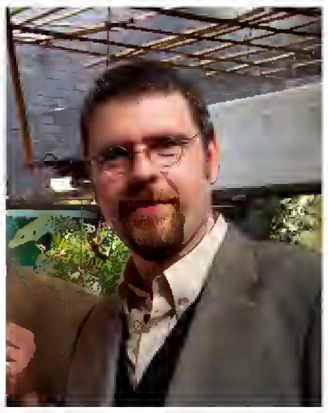
RIKE BACH, diploma biologist from Bonn, conducted her thesis in the ichthyology section of the Zoologisches Forschungsmuseum Alexander Koenig, Bonn. During her education at the Rheinische Friedrich-Wilhelms-Universität in Bonn she attended to a research project in the Cologne Zoo where she documented the early developmental stages of *Megophrys*. Her previous research was focused on the evolutionary biology of aquatic vertebrates in Southeast Asia.



DETLEF KARBE has been an employee of the Cologne Zoo since 1974 where he has worked for 20 years as gardener in the Aquarium / Terrarium Department. He is now a zoo keeper with his main focus of work on the construction of amphibian facilities and husbandry and breeding of anurans and salamanders. He has been involved in the successful breeding of *Megophrys nasuta* at the Cologne Zoo (and its respective record keeping), and in the breeding of threatened amphibian species such as *Atelopus flavescens* and *Tylototriton shanjing*.

KARIN VAN DER STRAETEN has been an employee of the Cologne Zoo since 1970 and is head keeper in the Terrarium Department. She is a zoo keeper focusing on amphibians and reptiles and during her career she has successfully bred more than ten species of amphibians.

STEFAN T. HERTWIG is the Head Curator of the Department of Vertebrate Animals at the Naturhistorisches Museum der Burgergemeinde Bern, Switzerland, and lecturer at the University of Bern. He studied biology and finished his Ph.D. at the Institute of Systematic Zoology, Friedrich Schiller University Jena, Germany. His scientific interests focus on biodiversity, phylogeny, and evolution of the frogs of Southeast Asia.



THOMAS ZIEGLER has been the Curator of the Aquarium / Terrarium Department at the Cologne Zoo since 2003 and is the coordinator of the Zoo's Biodiversity and Nature Conservation Projects in Vietnam. He completed his Ph.D. in the year 2000 at the Mathematical Scientific Faculty of the Rhineland Friedrich Wilhelms University Bonn focusing on the amphibian and reptile community of a lowland forest reserve in Vietnam. Since 1994, he has published 246 papers and books, mainly dealing with herpetodiversity. Since 2008, Thomas has been a member of the IUCN/SSC Amphibian Specialist Group within the Mainland Southeast Asia Region. His main research interests include diversity, systematics, and zoo biology of Southeast Asia's herpetofauna, in particular amphibians, monitor lizards, snakes, and crocodiles. Since February 2009, he has been Associate Professor at the Zoological Institute (Biocentre) of Cologne University.

Copyright: © 2012 Gawor et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Amphibian & Reptile Conservation 5(3):29-44.

Is there a chance for conservation breeding? *Ex situ* management, reproduction, and early life stages of the Harlequin toad *Atelopus flavescens* Duméril & Bibron, 1841 (Amphibia: Anura: Bufonidae)

[1,3]Anna Gawor, [1]Anna Rauhaus, [1]Detlef Karbe, [1]Karin Van Der Straeten, [2,4]Stefan Lötters, and [1,5]Thomas Ziegler

[1]*Cologne Zoo, Cologne, GERMANY* [2]*Trier University, Biogeography Department, Trier, GERMANY*

***Abstract.*—We report on our experiences with the captive management and *ex situ* reproduction of the Harlequin toad from Suriname (*Atelopus flavescens*) at the amphibian breeding unit of the Cologne Zoo. Egg deposition was stimulated by maintaining *A. flavescens* in a drier environment followed by a period of intensive irrigation. Here we provide for the first time an overview of the larval development from oviposition to metamorphosis, including diagnostic morphological characters according to Gosner. Eggs were arranged in strings and attached to the substrate below the water surface. Larvae hatched about five days after egg deposition and the characteristic abdominal suctorial disc developed about two days later (stages 20-21). Tadpoles are gastromyzophorous and were observed rasping algae. The average time for larval development to stage 41 was 100-130 days. Larval development appears to be dependent on water temperature with faster development at higher temperatures. Concerning color pattern in adults, we observed a slight sexual dimorphism and we were able to recognize individuals due to a constant color pattern. However, color was observed to slightly change over time.**

Key words. Anura; Bufonidae; *Atelopus flavescens*; husbandry; breeding; development; larval stages; adult color pattern; individual recognition

Citation: Gawor A, Rauhaus A, Karbe D, Van Der Straeten K, Lötters S, Ziegler T. 2012. Is there a chance for conservation breeding? *Ex situ* management, reproduction, and early life stages of the Harlequin toad *Atelopus flavescens* Duméril & Bibron, 1841 (Amphibia: Anura: Bufonidae). *Amphibian & Reptile Conservation* 5(3):29-44(e50).

Introduction

Harlequin toads of the bufonid genus *Atelopus* have a Neotropical distribution. They can be found in humid environments from Costa Rica south along the Andes stretch south to Bolivia and eastwards into the Amazon basin to eastern Guyana. This species-rich taxon is comprised of 113 taxa some of which are undescribed (La Marca et al. 2005). We are aware of additional new species, and taxonomic reviews of several *Atelopus* species complexes are still pending (e.g., Rueda-Almonacid et al. 2005; De la Riva 2011; Frost 2011). Many of these species have a highly restricted geographical distribution. This may be one reason why many *Atelopus* species are among the most hard-hit lineages in ongoing worldwide amphibian population declines and extinctions. *Atelopus* is one of the most threatened vertebrate groups in the world, with the majority of species having undergone dramatic declines within the last three decades. Many of these are so called "rapid enigmatic declines" and several populations and species are now thought to be extinct (La Marca et al. 2005; Stuart et al. 2008). Multidisciplinary conservation strategies are urgently needed (Lötters 2007). *Atelopus* species reproduce in streams and have rheophilic larvae. But apart from this, natural history information is sparse to lacking for most *Atelopus* species (Lötters 1996; Rueda-Almonacid et al. 2005; Karraker et al. 2006; Luger et al. 2009).

Many of the *Atelopus* declines and extinctions are presumably related to the occurrence of the amphibian fungal disease chytridiomycosis (Bonaccorso et al. 2003; Pounds et al. 2006; Lötters et al. 2010), which can occur even in undisturbed environments. As pointed out by Lötters (2007), *ex situ* conservation action, namely conservation breeding, should be considered among the potential measures to rescue these amphibians. This is in agreement with recommendations in the *IUCN Amphibian Conservation Action Plan*, which cites conservation breeding as an option for protection of many amphibians (see also Griffith and Pavajeau 2008; Browne et al. 2011; Lötters et al. 2011a; Zippel et al. 2011). Nevertheless, so far there are only few reports about successful captive breeding and rearing of *Atelopus* species (e.g., Mebs

Correspondence. *Email:* [3]*anna_gawor@gmx.de* [4]*loetters@uni-trier.de* [5]*ziegler@koelnerzoo.de* (corresponding author).

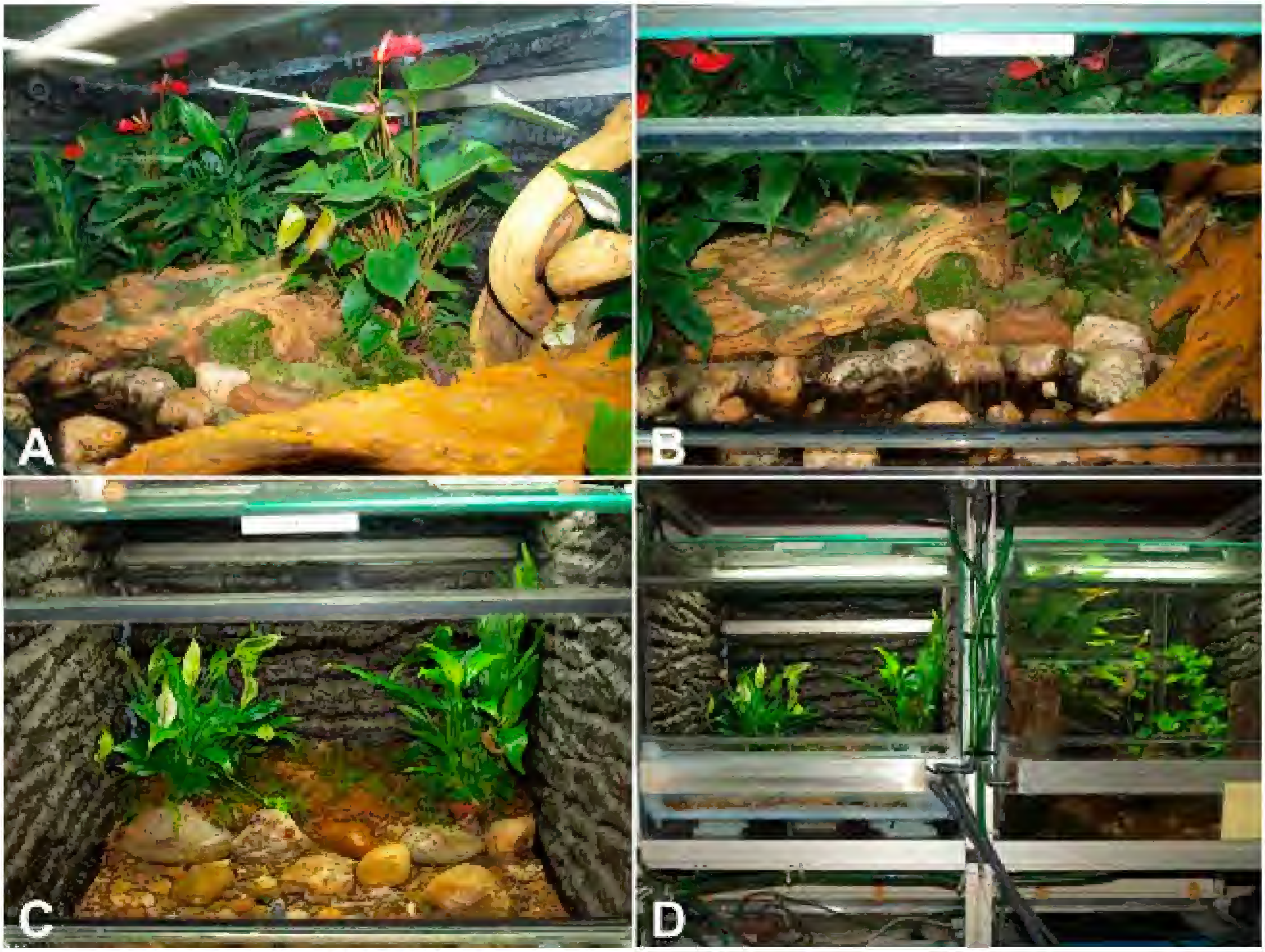


Figure 1. *Atelopus flavescens* terraria in the amphibian breeding unit at the Cologne Zoo from different perspectives (A) - (D); both terraria have artificial streams in the foreground. *Photographs by D. Karbe.*

1980; Heselhaus 1994; Haas 1995; Poole 2006; Siavichay et al. 2011). Likewise, little is known about *Atelopus* reproductive ecology in the wild (Karraker et al. 2006). Thus, it is not only important to widen the number of successfully bred *Atelopus* species, but also to report about any progress in breeding, and to better understand *Atelopus* reproductive biology and *ex situ* management for conservation breeding programs.

It is important to learn more about the reproductive biology and *ex situ* management of *Atelopus* as a basis for the further development of conservation breeding programs. For this purpose, we selected the Harlequin toad (*Atelopus flavescens*; Alonso and Mol 2007) from the Nassau Plateau and its vicinities in Suriname. It was discovered in 2007 and was identified as a color morph of the widely distributed polymorphic *A. flavescens* Duméril and Bibron, 1841 from the eastern Guiana Shield (Lötters et al. 2011b; S. Lötters and colleagues, data not shown). This species is one of the few apparently yet "intact" Harlequin toad taxa with stable populations (Rueda-Almonacid et al. 2005) and is occasionally available via the pet trade. We selected *A. flavescens* as a husbandry analogue species for the threatened genus *Atelopus*; to start with a relatively easy-to-obtain-taxon, which has relatively stable status in nature, and that is suitable for learning more about the husbandry and breeding of *Atelopus* species in general. About six years ago, Cologne Zoo (Germany), together with other European zoos (e.g., Zurich Zoo, Switzerland) and Atlanta Botanical Garden, established a cooperative conservation breeding program. To optimize *ex situ* conditions and to maximize captive reproduction success, field research has also been conducted (Lötters et al. 2011a). Data obtained from field studies finally led to successful *ex situ* deposition of eggs and subsequent larval development of *A. flavescens*. Herein we present our first experiences with the captive management and *ex situ* reproduction of *A. flavescens* at the amphibian breeding unit of the Cologne Zoo with emphasis on a description of mating, egg laying, and larval development.

Material and methods

In December 2006, Cologne Zoo received 15 *A. flavescens*, which originated from the vicinity of the Nassau Mountains, Suriname, from the Atlanta Botanical Garden for developing the international conservation breeding program. As all individuals turned out to be male, an additional group of 25 males and five females was obtained from the pet trade in December 2008. These animals were probably also derived from Suriname.

To provide vouchers, and to enable further study, some deceased adults were fixed in 40-60% ethanol and subsequently preserved in 70% ethanol and deposited in the herpetological collections of the Department of Herpetology and Ichthyology, Muséum d'histoire naturelle (MHNG), Geneva, Switzerland, and of the Zoologisches Forschungsmuseum Alexander Koenig (ZFMK), Bonn, Germany: MHNG 2727.25-2727.26 (n = 2), ZFMK 92947-92949 (n = 3). In addition, four freshly dead larvae in different developmental stages were fixed in 4% formalin and subsequently preserved in 70% ethanol. The larvae were deposited in the herpetological collection of the ZFMK (ZFMK 92351, deceased 22 December 2010, from first clutch 17 days after egg deposition, stages 24-25; ZFMK 92352, deceased 26 December 2010, from first clutch 21 days after egg deposition, stage 25; ZFMK 92353, deceased 29 December 2010, 24 days after egg deposition, stage 25; ZFMK 92354, deceased 26 December 2011, from second clutch 10 days after egg deposition, stages 22-23).

In addition, one deceased froglet (ZFMK 92350, from the first clutch; deceased 26 April 2011 at day 142, stage 46) and three malformed larvae (ZFMK 92955, deceased 22 December 2010, 17 days after egg deposition) were likewise fixed and preserved. Each preserved tadpole was used for closer character state examination and larval stage determination under a Leica binocular microscope.

After arrival, all adults were immediately photographed in dorsal and ventral views to examine whether individuals could be recognized using their distinctive color patterns. Egg clutches and larvae were photographed daily for documentation of their development. For assignment of developmental stages following Gosner (1960), as reproduced in Altig and McDiarmid (1999), several larvae were temporarily placed in glass vessels and photographed in dorsal, lateral, and ventral views. All photographs were taken with a digital camera (OLYMPUS E-600, DG MACRO 105 mm 1:2:8 object lens, SIGMA).

Abbreviations used are as follows: GH - total hardness, KH - carbonate hardness of water; pH - pH value of water; SVL - snout-vent length; TL - total length of tadpole. Terminology of larval morphology followed Altig and McDiarmid (1999).

Captive management of adults

After six weeks of quarantine, during which specimens were tested and found to be negative for the amphibian chytrid fungus (among other treatments), adult males were permanently maintained at Cologne Zoo in three groups consisting of 12 to 15 individuals in terraria measuring L100 × W60 × H60 cm. The five females were kept together in a terrarium measuring L60 × W45 × H40 cm, as in their natural environment, males and females occupy separate habitats throughout most of the year.

In the native environment, males stay in the vicinity of streams for longer periods or permanently (by implication, Kok 2000; Lötters et al. 2011a), while females have only been found inside the forest at least 25 m away from the closest stream. Females might appear at streams only shortly before mating. Back and side panels of the terrarium were pasted up with structure rear panels (Juwel) for providing a naturally looking environment. In male terraria, floor drains were installed and an artificial stream was constructed, which measured between 15 and 20 cm in width.

The stream was separated from the terrestrial part of the terrarium using 12 cm high glass strips pasted in with silicone. Different elevation levels were created using plastic light grid pieces, which were covered with one cm foam plastic and afterwards set in concrete. In order to provide easy access between land and water parts, as well as to form elevated "calling spots," several stones were placed in the stream before the concrete dried. Subsequently, smaller pebbles were brought in for a more naturalistic arrangement. To be able to reach the tubes of the filtration system and for cleaning, parts of the light grids were not set in concrete but only covered with pebbles. The total water depth in the terrarium was about 10 cm but the maximum depth accessible for the toads (measured from the concrete coat) was about three cm (Fig. 1 A-D).

An Eheim external filtration system (type: 2224, 50 Watt) with a capacity of 700 l/h was attached to the artificial stream. The water parameters were: pH = 7.12, GH = 6, KH = 3, conductivity = 280 µS, temperature = 22-24 °C. These parameters differ in some respects from those measured in the wild in *A. flavescens* stream habitats in French Guiana (Kaw, 7 July 1979: pH = 5, temperature = 25.5-26.0 °C (Lescure 1981); Noragues, 6 February 2010: pH = 6.5, GH < 1, KH < 1, temperature = 25 °C [P. Werner, data not shown]).

The terrestrial substrate in the terraria consisted of leaf litter, covered with forest moss in order to avoid pollution of the streams by ground substrate. A variety of plants (swamp grasses, small sized *Anthurium* sp. and *Spathiphyllum* sp.) completed the terrarium structuring.

Illumination was provided via T5 fluorescent tubes (males: Osram FQ, 865 Lumilux daylight: single-flame 36 Watt, females: dual-flame 24 Watt). The photoperiod lasted between nine and 12 hours; in addition, three room windows allowed for natural light and fluctuation of day lengths.

Daily average temperatures in the terraria measured between 24 and 27 °C throughout the year; the relative humidity ranged between 60 and 100%. In the beginning, terraria with males were fogged several times a day with a humidifier (Lucky Reptile SuperFog). After one year, all terraria were only sprayed once a day with a manual pump sprayer. In October 2010, a rain system (Namiba's Tropical Rainsystem) with a coarse nozzle insert (Gloria) was installed to amplify the former manual irrigation. The rain system was run five or six times a day for 10 to 20 minutes; about 10 liters of water per 15 minutes were sprayed. At night, no irrigation took place.

Terrarium for egg deposition

Females were transferred to one of the male terraria, which soon led to the first egg deposition within the stream bed (see Results). For better observation, another, completely water-flooded terrarium with rocks breaking through the surface (L60 × W60 × H55 cm) was prepared, intended for subsequent concerted separation of couples for mating. Here, a second egg deposition took place (see Results). A connected adjacent aquarium, equipped with three foam mats and with a capacity of 90 liters (L60 × W60 × H25 cm) served as an external filter. In addition, a constant drop-wise fresh water supply was attached. The water temperature was maintained at about 24 °C by the use of a filter heater.

Plastic light grids were laid out over top of the filtration tubes in order to achieve a maximal water depth of six cm at a water volume of about 36 liters and to hide the filtration system tubes. The light grids were covered with filter fleece, a thin layer of river sand (particle size: 0.2 mm) and several pebbles; the edges of the fleece were sealed with aquarium silicone to prevent the tadpoles from escaping below the ground cover. In the back part of the terrarium, a small artificial shore zone was constructed by layering pebbles and moss. The same type of rain system as used for the male terraria was installed for irrigation. The rain system was run four times a day for 15-30 minutes; during night time, no irrigation took place.

Captive management of larvae

The larvae of the first clutch were left in the artificial stream within the terrarium of the adult males. For maintaining constant water parameters, fresh water was supplied (ca. one drop per second), the first five days for three hours a day and later, constantly. The last surviving tadpole was later transferred into a small gauze aquarium (L16 × W10 × H10 cm), which was suspended into a larger aquarium (L119 × W43 × H30 cm) with the following water parameters: pH = 7.12, GH = 1, KH = 1 or 2, conductivity = 206 µS, temperature = 22.8 °C. An external filter with a capacity of 500 l/h and a universal water pump (Eheim, 600 l/h) was attached. Illumination was provided by a T5 fluorescent tube (Osram FQ, 865 Lumilux daylight: single-flame 54 Watt), which was mounted 70 cm above the water surface. To allow the metamorphosing froglet to leave the water, a ramp of pebbles was placed in one corner of the gauze aquarium.

The larvae of the second clutch remained in the tank that was erected for egg deposition, but in contrast to the first clutch, adult individuals were not housed in the same tank.

Nutrition

Adults were fed two or three times a week during their activity time (daytime); the food consisted of small invertebrates, including fruit flies (*Drosophila* sp.), small house crickets (*Acheta domestica*), and springtails (Collembola). All insects were nourished with high quality food and dusted with mineral and vitamin supplements (Korvimin ZVT + Reptil/Calcamineral) before being fed.

Tadpoles were fed with algae growing on the stones in the artificial streams. In addition, different sorts of fish food (*Spirulina*-tabs, *Spirulina*-powder, Sera-flora, algae-chips) were offered. The fish food was pulverized, mixed with water, applied to flat stones, and inserted into the stream bed after drying.

Results

Pre-mating observations and mating

Throughout the year, males showed calling activity after the daily spraying of the terrarium (Fig. 2 A, B). From the end of September until the end of March or beginning of April the calls occurred more frequently than during the rest of the year, and also occurred beyond the irrigation periods, mostly in the morning. Usually the male that was thought to be the dominant individual in the group, started the calling activities. Individuals could be identified by their characteristic back patterns.

In March 2010, two females each were introduced into two male groups. Three of the females were observed in amplexus after being introduced. The fourth female averted all mating attempts of the males and was removed from the terrarium after four weeks. The axillary amplexus lasted for about five weeks (Fig. 2 C) and the involved males did not appear to feed during this time. After the couples had split up without egg deposition, the three females were removed from the male terraria. Two further trials in May and June 2010 also led to amplexus but without oviposition.

Afterwards, a dry season with reduced water level and minimal spraying was induced. The males discontinued calling and reduced their food intake and their daytime activity by remaining stationary on elevated leaves or under the moss pads. Their legs were often held tightly against their bodies. After three months of dry season (July until September), at the beginning of October 2010 (simulating the small rainy season in the species' natural habitat), a wet season with intensified manual spraying and a higher water level was initiated.

In mid October, one female each was introduced to a male group (with all individuals coming from the second group, received in December 2008). After about three weeks, amplexus took place with both females. From 29 November 2010, the previously introduced rain system was used to amplify the irrigation. During and after the irrigation, all the males were highly active, showing calling activity and preferring to be exposed to the rain, while the couples in amplexus searched for hiding places. At 5-10 minutes after the irrigation, the couples came out and often stayed within the stream. The solitary males frequently importuned the couples in amplexus; one time a male was observed pushing a couple under water for about five minutes.

On 2 December 2010, no irrigation was effected; the next day, the rain system was only run two times, once for five and once for 10 minutes. On 4 and 5 December, again no irrigation was induced. The first oviposition took place during the night from 5 to 6 December, shortly after the reduction of the intensive irrigation. About six weeks later, in the night from 16 to 17 January 2011, a second oviposition occurred, but this time not in the males' terrarium but at the terrarium especially prepared for egg deposition. A few days before, on the 6 January,

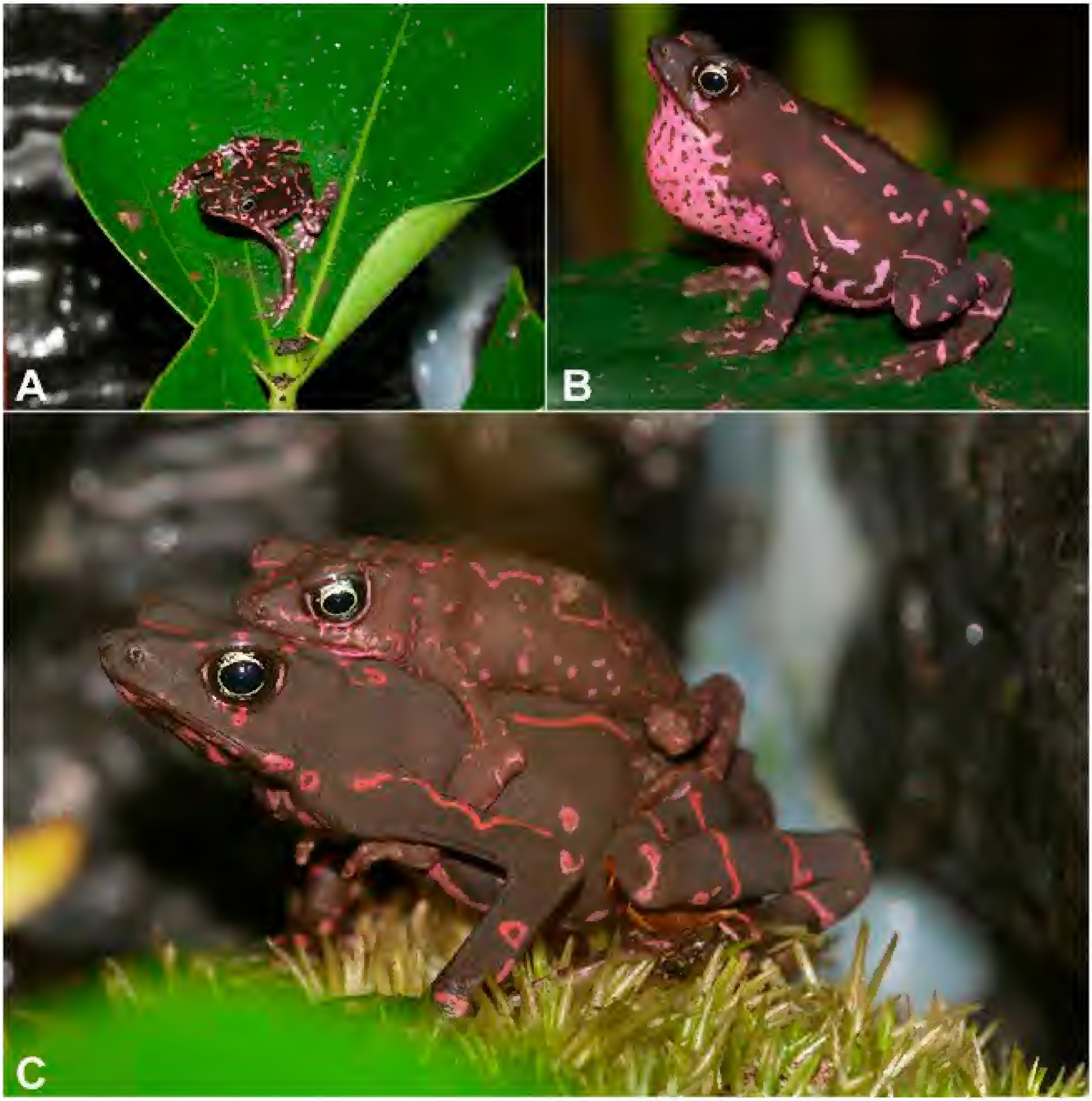


Figure 2. *Atelopus flavescens* at the amphibian breeding unit at the Cologne Zoo: (A) adult male, (B) calling male, and (C) couple in amplexus. *Photograph* (A) (B) *by T. Ziegler and* (C) *by D. Karbe.*

four males had been placed in this terrarium, joined by a female from 10 January onwards. At that time the irrigation system was turned on constantly for 30 minutes daily. Amplexus took place one hour after the female was introduced. The irrigation frequency (see Material and methods) remained unchanged until the oviposition event.

Clutch deposition and description

The first deposited egg clutch (December 2010) consisted of more than 500 eggs, which were arranged in single strings, partly branched (i.e., peripheral rami), and affixed about two cm under the water surface to stones or filamentous algae. The cream-colored eggs (ca. one mm in diameter) were surrounded by a thin membrane and a gelatinous capsule (total diameter ca. three mm) (see Table 1, Fig. 3 A, B). On the third and fourth day after egg deposition, a consistent clockwise rotation of the eggs could be observed; on the fifth day the rotation of the eggs changed direction and started moving counterclockwise. The smallest egg-string (containing 27 eggs) was found to be unfertilized on the fourth day after egg deposition while the other eggs showed discernible development (Fig. 3 C, D).

In contrast to the first egg deposition, the second egg deposition, which took place during the night from 16 to 17 January 2011, occurred under the hollow of a large stone. Before egg-laying, the couple had shoved aside smaller pebbles from the deposition place. The clutch consisted of more than 400 eggs of about the same size as in the first clutch, and of which ca. 10% were unfertilized.

Two deceased adult females contained large yellowish-orange eggs: ZFMK 92947 (SVL 33.6 mm) had eggs with 1.2 mm maximum diameter; ZFMK 92948 (SVL 30.2 mm) had eggs with 1.3 mm maximum diameter.

Larval development and stages

Hatching of tadpoles from the first clutch started seven days after egg deposition (12 December 2010). All larvae hatched during the night and were found next to the eggs the next morning where they remained for the following days; first movements of the tails could be noticed on the day after the hatch (stage 20). The larvae had a total

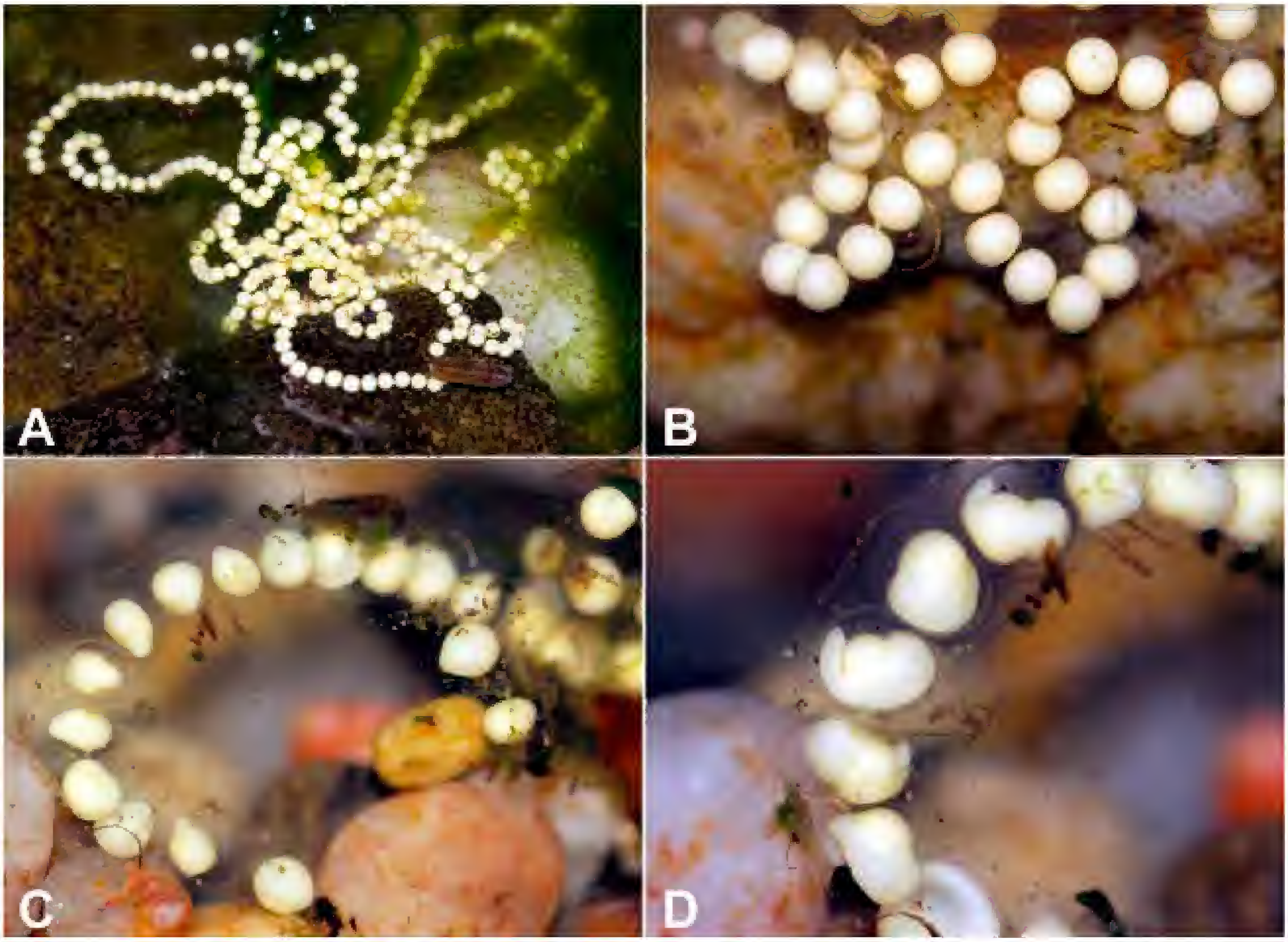


Figure 3. First clutch of *Atelopus flavescens* at the amphibian breeding unit at the Cologne Zoo: (A) freshly deposited spawn under water surface on stones or filamentous algae (5 to 6 December 2010), (B) cream-colored eggs one day after deposition (6 December 2010), (C) developing embryos at Gosner stage < 18 (9 December 2010), (D) embryos at stage 19 (10 December 2010). *Photographs by D. Karbe.*

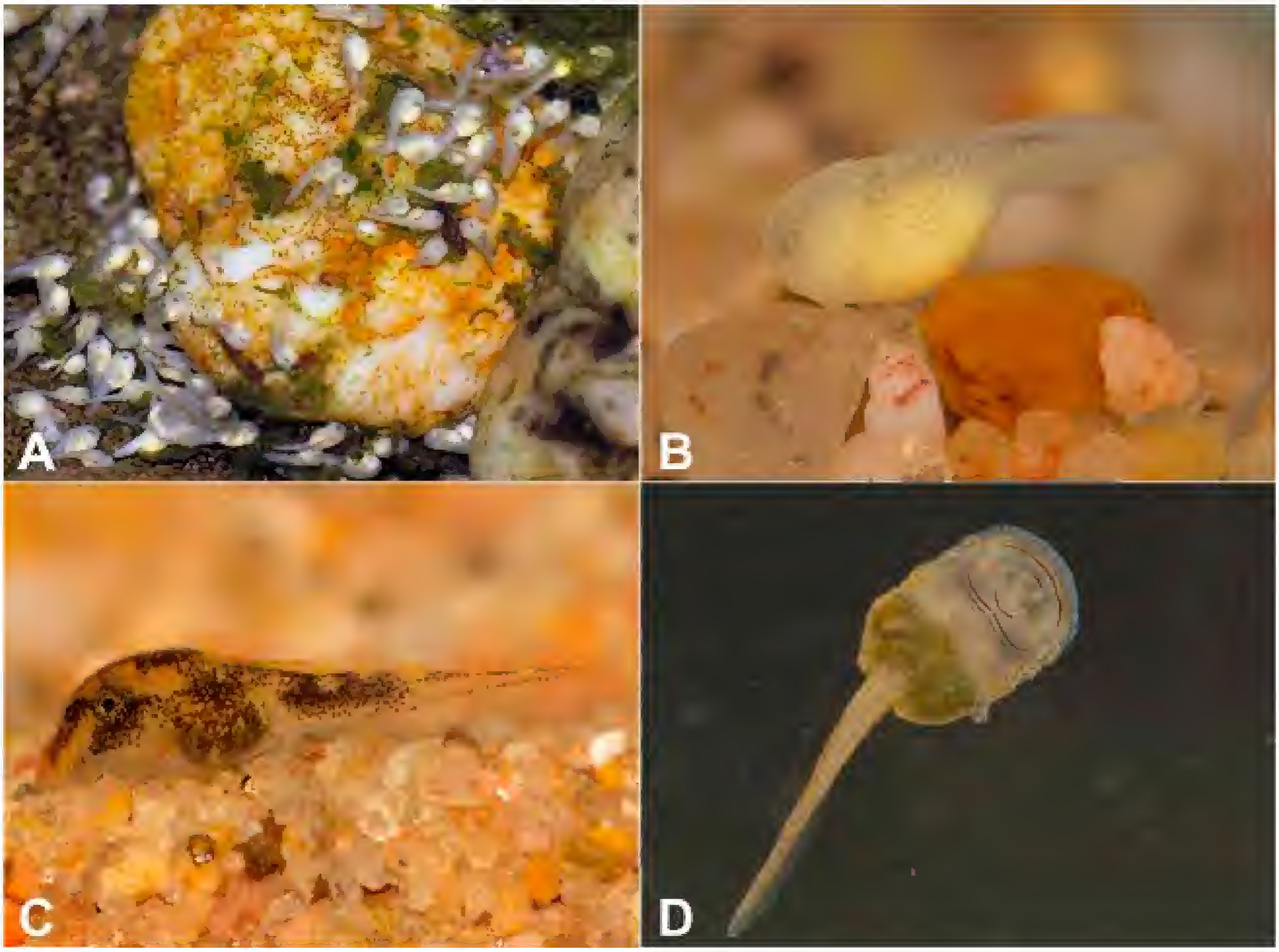


Figure 4. Hatched larvae of *Atelopus flavescens* (from first egg deposition): (A) - (B) hatchlings at Gosner stage 20 (13 December 2010), (C) lateral view of tadpole at stages 24-25 (27 December 2010, 22 days after egg deposition), (D) ventral view of tadpole at stage 25 (3 January 2011, 29 days after egg deposition). *Photographs by D. Karbe.*

length of 3.9 mm and a tail length of 1.9 mm. We noticed dark pigmentation in the form of irregular blotches, reaching from the lateral and dorsal sides to the tail region. The ventral side lacked pigmentation. The prospective eye region was already visible at this stage (Fig. 4 A, B). Ten days after egg deposition (stage 21), the abdominal suctorial disc was discernible. The nostrils were indicated by two white spots, the developing eyes by two black spots. The lateral and dorsal blotches darkened and expanded to the ventral side. On day 14 (stages 21-23), the first larvae were seen swimming. One day later, most of the larvae were well distributed over the available space; they covered short distances swimming and adhered themselves to the substrate with their abdominal suctorial disc, which now covered three fourths of the ventral side. The yolk reservoir was completely absorbed and the oral disc was not completely developed. Sixteen days after egg deposition first feeding was observed (stage 24-25, see Fig. 4 C, D). Tadpoles were able to stay adhered while moving and feeding, classifying them as belonging to the gastromyzophorous type of rheophilic anuran larvae (Altig and McDiarmid 1999).

Tadpoles fed on algae growing on the stones in the artificial streams. However, we could not confirm uptake of the pulverized and mixed fish food that was provided. The dark pigmentation increased forming connected blotches. In addition, several golden spots showed up at the dorsal body side. In some of the larvae, the eyes were well discernible and the vent tube could be distinguished for the first time. The vent tube, which measured about 0.1 mm at this time, grew longer during development and showed a golden spotted coloration from day 23. Depending on the lighting, the heart was visible under the skin surface.

Twenty-two days after egg deposition, first excretion of feces could be detected. Twenty-five days after egg deposition, a tadpole at stage 25 was carefully inspected under a binocular microscope. Here, papillae at the edges of the oral disc, which covered more than two thirds of the ventral side at this time, were discernible, as well as the tooth rows in the oral disc (labial tooth row formula was 2/3). The body surface was covered with a large number of golden spots; the dark ventral pigmentation had reduced to smaller, isolated blotches. Twenty-six days after egg deposition, intestines were visible. On day

Table 1. Developmental stages of *Atelopus flavescens* bred at the Cologne Zoo from Gosner stage 1 to the completion of metamorphosis including diagnostic features according to Gosner (1960); TL = Total length (mm), labial tooth formula = number of tooth rows per upper/lower labium, SVL = snout-vent length (mm); water temperature = 22-24 °C; (1) = larger tadpole, (2) = smaller tadpole, as explained in text.

Age (days)	Gosner stage	Diagnostic features	
1	**1-12**	egg clutch arranged in branched strings; eggs cream-colored; diameter of single egg without transparent jelly capsule about 1 mm	**Embryonic stages**
2-5	**13-19**	embryos assume larval shape with head region set off from tail; yolk reservoir present; larvae uniform yellowish	
7-8	**20**	larvae hatched; elongation of body and tail; development of recognizable head; formation of greyish pigmentation pattern begins on upper region of head, body and tail; tail fins become transparent	**Hatchlings**
10-15	**21-23**	free-swimming larvae: tail longer than body; body ovoid in dorsal view, laterally depressed; increase of pigmentation on body and tail; eye region begins to develop; nares present; spiracle sinistral, laterally situated; oral disc differentiation begins; abdominal suctorial disc extending from posterior labium until half of body; vent tube present; yolk reservoir absorbed on day 15	
16-43	**24-25**	feeding tadpoles: TL > 5.0 mm: golden blotches on body and tail appear; eyes clearly discernable; oral apparatus completely developed on day 22: upper and lower beak slightly keratinized to distal edge, labial teeth present (labial tooth row formula 2/3), upper labium with marginal papillae; abdominal suctorial disc rounded, extending from posterior labium for more than half the body length; elongation of spiracle; intestinal coils visible through integument > day 26, stage 25	
46	**(1) 26**	(1) TL > 7.0 mm; appearance of hind limb buds in larger tadpole	**Larvae – Metamorphosis**
65	**(2) 26**	(2) TL > 7.0 mm; appearance of hind limb buds in smaller tadpole	
75-79	**(1) 28**	(1) TL > 10.0 mm; length of hind limbs ≥ basal width	
83	**(1) 30** **(2) 27**	(1) length of hind limbs = two times basal width; appearance of pigments on hind limbs; (2) length of hind limbs ≥ one half basal width	
86	**(1) 31**	(1) ongoing developing of limb buds: foot paddle shaped	
90-95	**(1) 33-34**	(1) development and differentiation of toe 2-4	
97-101	**(1) 36-37** **(2) 28-29**	(1) development and differentiation of toe 1-2, begin of toe separation; pigmentation of hind limbs darkens; forelimbs visible through integument > day 101; atrophy of vent tube; (2) length of hind limbs ≥ one half basal width	
103-106	**(1) 37-41;**	(1) mouthparts and abdominal suctorial disc atrophy; spiracle still present; changes of metamorphosis begin; disappearing of tadpole on day 112	
109	**(2) 34**	(2) toes development	
119-122	**(2) 36-37**	(2) TL > 13.0 mm; growing and separation of toes (toes completely separated on day 122); forelimbs visible through integument	
129-130	**(2) 40-41**	(2) changes of metamorphosis begin: mouthparts, abdominal suctorial disc and spiracle atrophy; vent tube gone; tail atrophy begins; forelimbs pigmented, increased in length	
131	**(2) 42**	(2) forelimbs emerged; mouth anterior to nostril, tail mostly reduced	
133-134	**(2) 43-44**	(2) mouth between nostril and eye; tail greatly reduced	
139-140	**(2) > 46**	(2) SVL 6.0 mm; tail resorbed; forelimbs malformed	

30, we noticed a large decrease in the number of larvae in the stream, but no dead larvae were found.

On day 43 after egg deposition, only two larvae were detectable in the stream. Both were in different developmental stages and later died at different stages. In the following, we first describe the development of the larger larva from day 43 onwards (see Table 1, Fig. 6), and subsequently the development of the smaller larva.

On day 46 after egg deposition, the larger larva began to develop hind limb buds (stage 26). After 75 days (stage 28), this larva measured 10 mm total length (TL). The hind limb buds were clearly visible at this time (Fig. 5 A). On day 83 (stage 30) dark pigmentation had developed on the hind limb buds. These were followed by golden spots, which appeared at day 89, and a rust brown coloration appearing four days later. Development of toes began at day 90. Five days later (stages 33-34), the coloration of the spots on the body surface partly turned from golden into a rusty brown. On day 97 (stages 36-37), separation of toes started. After 101 days, developing forelimbs were visible under the skin surface. From day 105 (> stage 39), hind limbs were actively used to support locomotion and from day 112 on, the development of this tadpole could not be documented anymore as it disappeared (and apparently died).

Sixty-five days after egg deposition, the smaller larva began to develop hind limb buds (stage 26, a stage which had been reached by the aforementioned larger larva already 19 days before, i.e., 46 days after egg deposition; see Table 1). On day 75, this tadpole measured seven mm TL, and on day 100, slightly pigmented hind limb buds were clearly visible without the use of a hand loupe

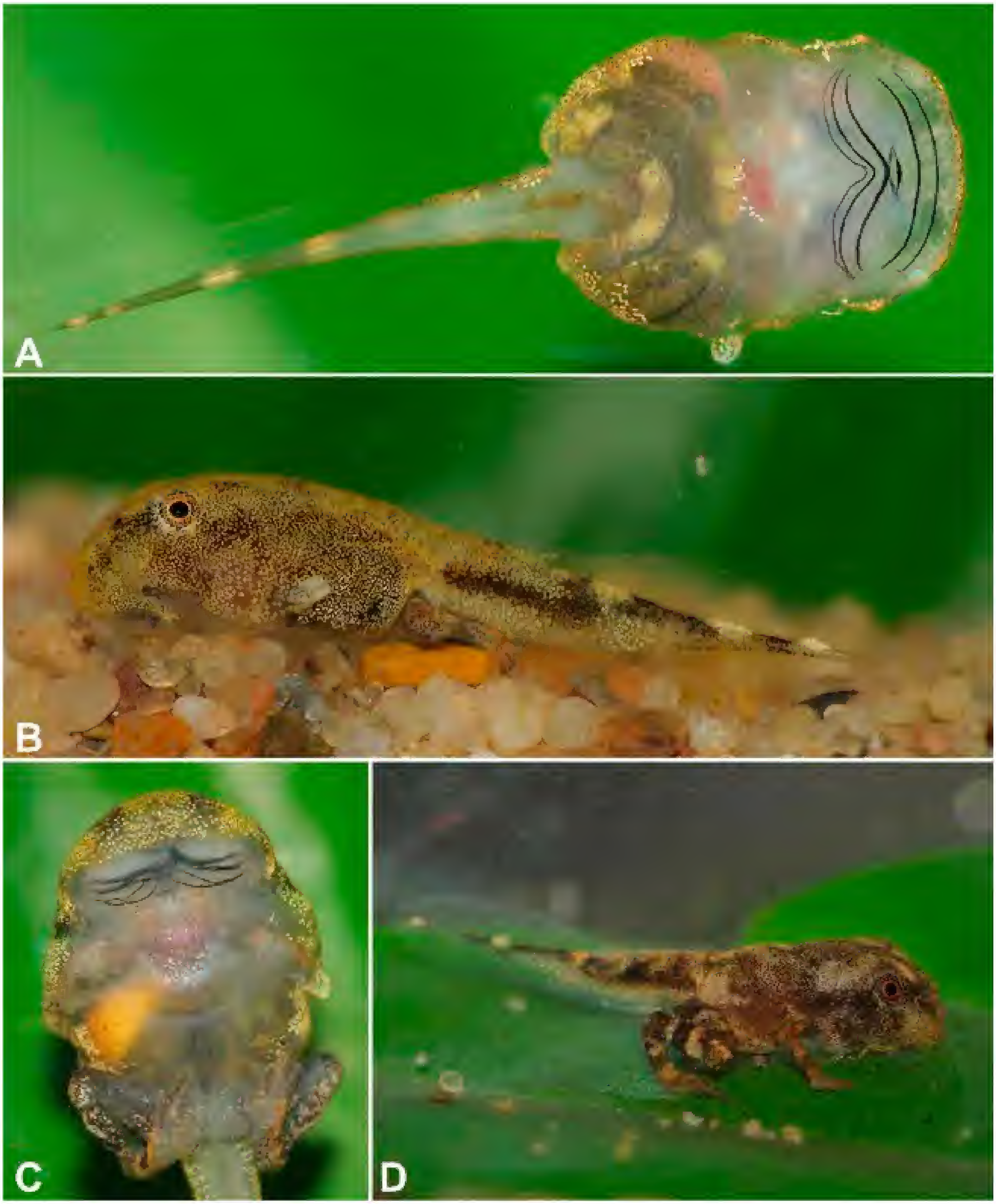


Figure 5. Tadpoles of *Atelopus flavescens*: (A) ventral view of larva at Gosner stage 28 (22 February 2011, 79 days after egg deposition; from first clutch; larger larva), (B) lateral view of tadpole at stages 34-36 (22 April 2011, 96 days after egg deposition; from second clutch), (C) ventral view of tadpole at stage 41 (26 April 2011, 100 days after egg deposition; from second clutch), (D) tadpole at stage 42 (15 April 2011, 131 days after egg deposition; smaller larva). *Photographs by D. Karbe.*

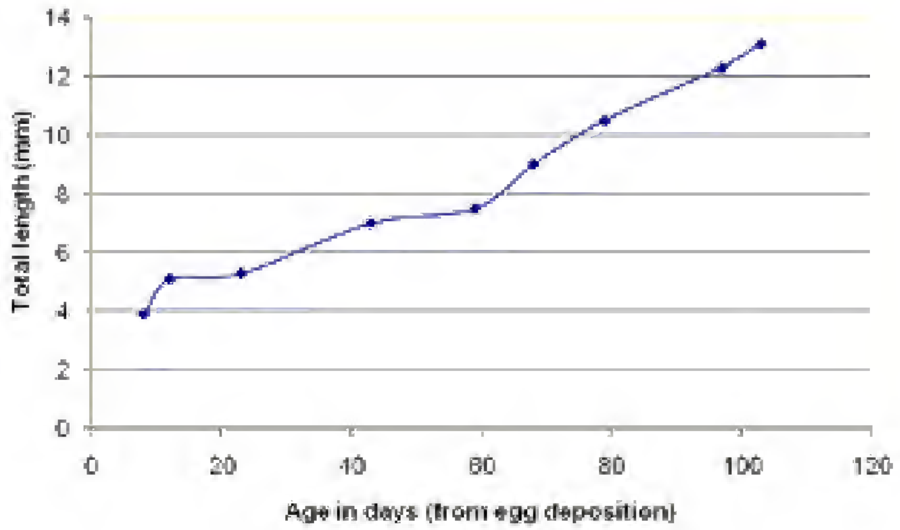


Figure 6. Total length (mm) of larger tadpole of *Atelopus flavescens* from first clutch in relation to age in days; water temperature 22-24 °C.

(stages 28-29). After 119 days (stages 36-37), this larva had reached TL 13 mm. Hind limbs, which were tightly attached to the tail at this stage, measured about 2.5 mm, and were rusty-brown in coloration. On day 122 the legs, with all toes being separated, could be moved and the fore limbs were already discernible. Two days later, the larva was transferred into a separate aquarium (see Material and methods). In order to provide food resources, some stones overgrown with algae were added. After 129 days (stages 40-41), the fore limbs were pigmented and well recognizable under the skin surface; the intestine was less distinct. At that time the tadpole remained near the stream substrate more frequently. The dorsal pigmentation gradually changed: the bigger blotches were still dark, while the coloration of the smaller spots turned

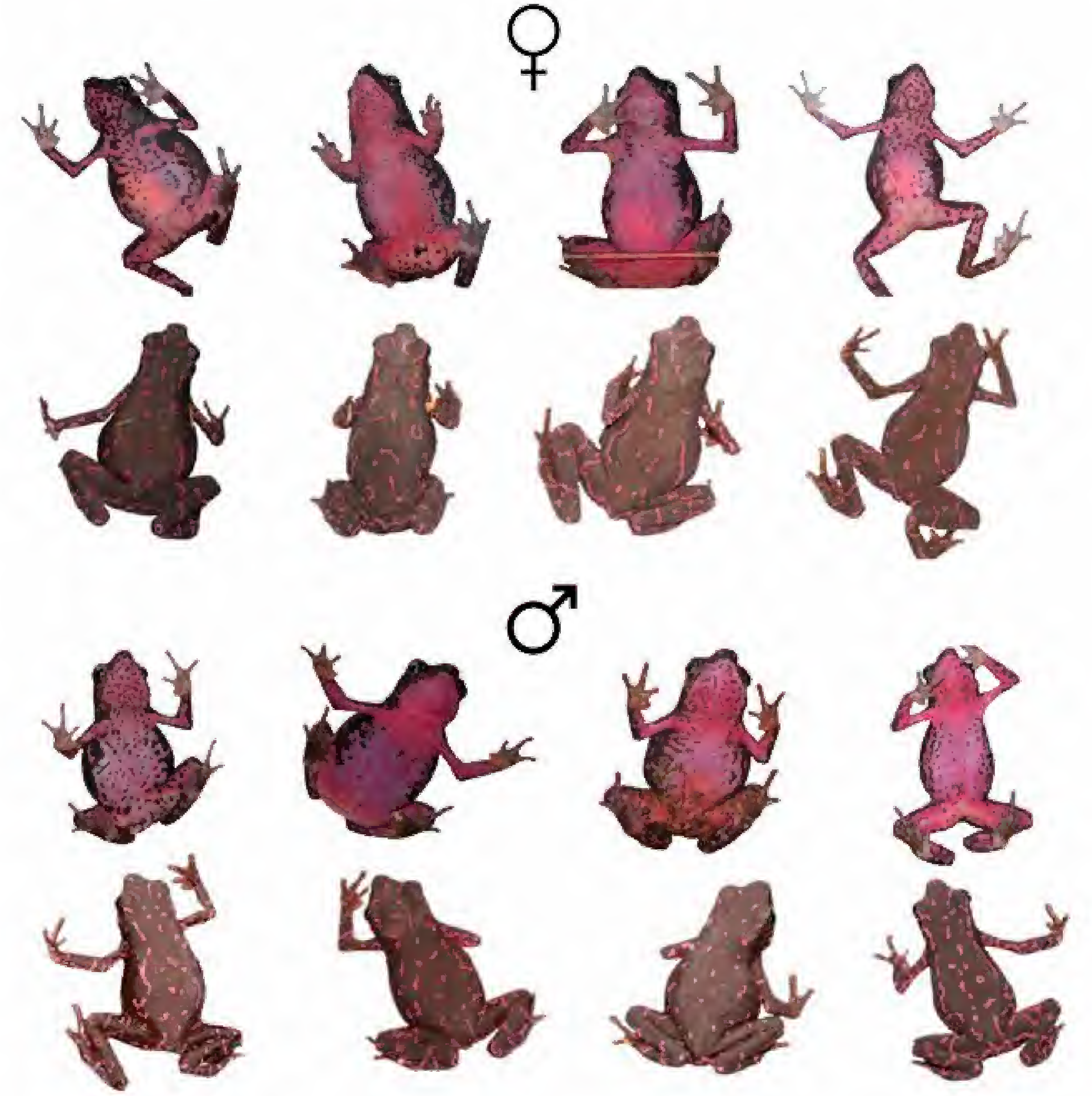


Figure 7. Color patterns of *Atelopus flavescens* at the amphibian breeding unit at the Cologne Zoo: Four females (above) and males (below) in ventral and dorsal views. *Photographs by D. Karbe.*

from golden into a yellowish taupe. The ventral side was partly transparent; the inner surface of the legs was dark pigmented, with several black spots. The soles of the feet were colored rusty brown. 131 days after egg deposition (stage 42, Fig. 5 D), the fore limbs started to protrude, but were malformed (the so-called spindly leg syndrome). One stuck out at a 90 degree angle and the other was angular and could not be stretched. The abdominal suctorial disc as well as the oral disc were reduced and had completely disappeared three days later; the tail also started to resorb.

At day 137 the froglet, which measured 6.0 mm SVL, tried to move out of the water and onto the land for the first time, but it could not stand erect due to the fore limb malformations. Two days later, the tail was completely resorbed (stage 46). Subsequently, no intake of the provided food (spring tails) could be observed. The froglet died at day 142 after egg deposition. Its color had not changed further by that time, i.e., the purple coloration of adults had not appeared by that time.

The development of larvae from the second egg deposition is summarized in Table 2. In this second reproduction phase, larval development could be observed until day 100 (stage 41) before the last tadpoles disappeared.

Individual recognition based on color pattern

By taking photographs of every adult individual and comparing them regularly, we observed that specimens maintained their individual color pattern. The dorsal pattern differed in number, arrangement, size, and shape of the pink-colored spots, stripes, and circles on a dark-brown background. The ventral pattern varied in the arrangement of irregular dark-brown to black blotches on a purple background (Fig.7).

Table 1. Comparison of developmental time (age in days) including stages according to Gosner (1960) between first reproduction phase (5 to 6 December 2010, water temperature 22-24 °C) and second reproduction phase (17 January 2011, water temperature 24 °C) of *Atelopus flavescens* bred at the Cologne Zoo.

Gosner stage	Age in days (first breeding)	Age in days (second breeding)
1-12	1	1
13-19	2-5	2-4
20-21	7-8	5-6
21-23	10-15	7-11
24-25	16-43	16-38
26	46 and 65	39
27-28	–	41
28-29	< 101	51-62
30-32	–	80
32-33	–	83
34-39	–	87-96 (Fig. 5 B)
41	> 106-130	100 (Fig. 5 C)

We also observed that the individual patterns did not change with age. Based on the comparison of photographs taken over two years we were able to determine that the pattern remained the same, but the dorsal coloration changed slightly from dark brown to dark grey or almost black, while the coloration of the spots, stripes, and circles turned from pink to yellowish-white over time (Fig. 8). We also observed a potential slight sexual dimorphism. Compared with the gular region of the females, the throat region of the males appeared to be more intensively purple-colored.

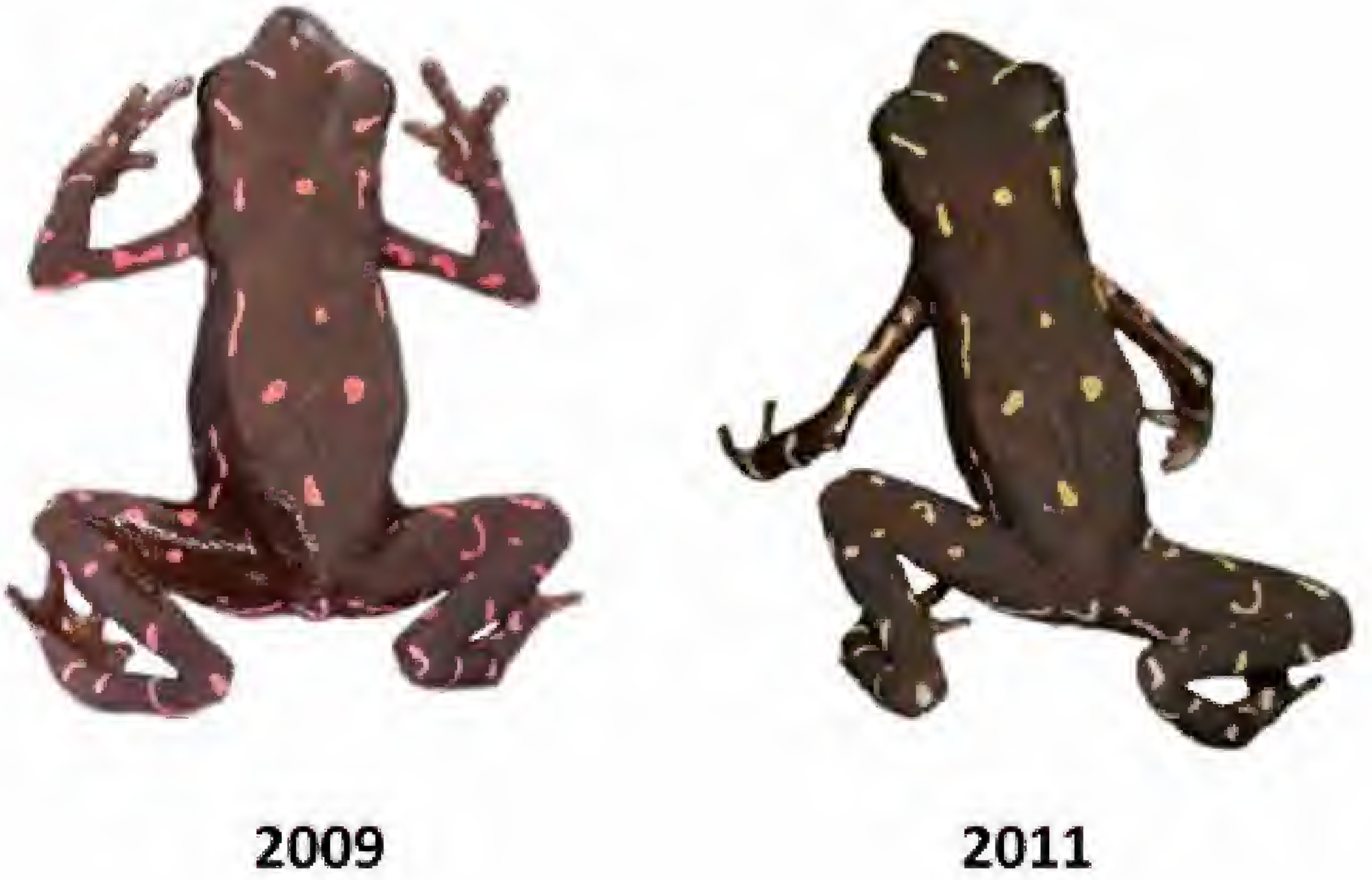


Figure 8. Individual recognition of a male *Atelopus flavescens* based on color pattern, but note the change in color (photographs taken 12 July 2009 and 31 July 2011, respectively). *Photographs by D. Karbe.*

Discussion

During the husbandry and breeding of *A. flavescens* at Cologne Zoo we identified a several months long dry period as a trigger for reproduction. This was done to mimic the dry season in the natural habitat, and was followed by a period of intensive irrigation. In the wild, *A. flavescens* reproduce with the beginning of rains (i.e., October/November to January; April/May to July; Lescure 1981; Boistel et al. 2005; Lötters et al. 2011a). As a reaction to the artificially induced drier period, the toads showed reduced activity, and we often observed them with their limbs closely pressed to their bodies. This posture was probably a reaction to the low humidity because the reduction of the body surface area minimizes water loss from evaporation.

There is little known about the reproductive phases in *Atelopus* species in the wild but the break of the short rainy season is apparently favored for breeding by several species. This may be explained by the fact that Harlequin toads breed in streams and that generally the risk of being washed away by the current is limited when rains are not too heavy (Lynch 1986; Lötters 1996; Karraker et al. 2006). This may be especially important in montane habitats. In lowland populations, like those of *A. flavescens*, it seems that all kinds of rains (with previous drier phases) can trigger species to start reproductive behavior as breeding apparently also takes place during the long rainy season (Boistel et al. 2005). As in the related Guianan *A. hoogmoedi* (Luger et al. 2009), *A. flavescens* males remain at streams in high density for most or all of the year, while females are found at larger distances from streams (Lötters et al. 2011a). Keeping the sexes separate from each other and introducing females to male groups may have triggered the toads to breed.

After increased irrigation, couples in amplexus came out of their hiding places and remained within the stream for some time. Because egg deposition did not take place immediately, and because we also observed the same couples in amplexus in different parts of the stream, we thought that the *A. flavescens* might have been searching for optimum oviposition places. Karraker et al. (2006) reported that in the Panamanian *A. zeteki*, oviposition sites were apparently carefully chosen. Prolonged amplexus, even for weeks, is common in *Atelopus* species and has been reported in wild populations of many species (Lötters 1996).

Whereas the first oviposition was done in the open water, the second oviposition took place below a larger stone. Such hiding places were missing in the stream environment within the first reproduction. Perhaps, shelter within the water body should be offered during captive management. Interestingly, Poole (2006) pointed out that *A. zeteki* eggs may show some light sensitivity. This needs further investigation, especially since Lescure (1981) found an *A. flavescens* clutch below, and Boistel et al. (2005) found one on top, of a rock in the wild. However, other *Atelopus* species apparently perform both oviposition on top of or below submerged rocks (Karraker et al. 2006).

A clutch of *A. flavescens* reported by Boistel et al. (2005) contained fewer eggs (ca. 250) than those obtained in captivity by us, but the clutch geometry was similar with several peripheral rami. These apparently function to stabilize eggs in the stream current and have also been reported in *A. subornatus* from Colombia (Lynch 1986), while in *A. zeteki*, Karraker et al. (2006) described egg strings to be “wrapped back up on themselves creating two or more layers.” Clutch size appears to be quite variable within and among *Atelopus* species, as summarized by these aforementioned authors.

Eggs known from other *Atelopus* species are similar in color but most of them are larger than those described here (Karraker et al. 2006) including those of *A. flavescens*. Lescure (1981) referred to an ovum diameter of > 1.5 mm versus ca. one mm only.

Larval stages of several *Atelopus* species have been described (e.g., Lötters 1996). Tadpoles obtained under captive conditions are consistent with those of *A. flavescens* collected in the wild (Lescure 1981; Boistel et al. 2005). In contrast, little information is available on larval development in Harlequin toads. Like in other species (summarized by Karraker et al. 2006), *A. flavescens* embryonic development is short (for comparisons, *A. cruciger* 3-4 days at 20 °C; *A. varius* six days at unknown temperature; *A. zeteki* 7-11 days at 22 °C) and hatchlings measure few millimeters only. Similar to observations by Karraker et al. (2006) on freshly hatched *A. zeteki*, the abdominal suctorial disc developed several days after hatching in *A. flavescens* (i.e., Gosner stage 21) allowing them to adhere to the substrate.

Regarding further larval change until metamorphosis, to the best of our knowledge, there is no information on other Harlequin toads for comparison. Only Lindquist and Hetherington (1998) described metamorphs of *A. zeteki* in Gosner stage 46 and older. They were larger (8.4-17.1 mm SVL) than the single specimen obtained by us. Similar to *A. zeteki,* freshly metamorphosed *A. flavescens* apparently have camouflage coloration rather than any brilliant colors.

In comparing larval development between the first and the second reproductive events, we observed slightly faster development (1-2 days) of larvae from the second egg deposition. This might be due to the more constant and somewhat higher water temperatures during the second reproductive event (24 °C) compared to the water temperatures of the first (22-24 °C).

In both reproductive events a noticeably large number of larvae disappeared. Similar observations were made by Heselhaus (1994) on *A. zeteki* (under the name *A. glyphus*) and Haas (1995) on *A. pulcher*. We cannot explain this. Because in our first reproductive event the adult males remained in the terrarium with the larvae, it cannot be ruled out that adults preyed on the tadpoles (see also

Poole 2006). However, such behavior was not observed during the daytime, and we consider cannibalism can be largely ruled out as *Atelopus* species are known as microphagous anurans feeding on land and preying on ants, mites, and termites (e.g., Lötters 1996).

In the terrarium for egg deposition, where larvae from the second reproductive event were maintained separate from adults, a few dead larvae could be found in the water (already eroded by snails). However, dead larvae never were found in the filtration system, which then would have been an indication that weak larvae might have been absorbed by the filtration system. Here, a possible reason for the abrupt decrease in numbers of larvae, assuming that the missing larvae had died, could be an insufficient oxygen concentration in the water (e.g., due to a shortage of current/air inclusion).

Dissolved oxygen in water is critical to larval development in *Atelopus*, including lowland species. Lescure (1981), Coloma and Lötters (1996) and Lindquist and Hetherington (1998) measured relatively high concentrations in the larval habitats of *A. flavescens*, *A. balios*, and *A. zeteki*, respectively. Lötters (1996) argued that due to their gastromyzophorous diet and occurrence in streams, tadpoles in later stages, when lungs have developed, only receive oxygen from the water through their skin. However, many of the tadpoles in our study disappeared in earlier stages and apparently coped well with oxygen conditions in the terrarium.

Another possibility may involve temperature or water chemistry, as pH, GH, and KH values measured during our efforts to rear *A. flavescens* tadpoles differed somewhat from those taken in a stream where this toad breeds in French Guiana (see above). Temperature was similar to that recorded in the wild, but differed from that measured by Boistel et al. (2005), which was only 20 °C.

Apart from this, changes in water conditions or a lack of food resources could represent possible causes for mortality. An argument for lack of food resources causing mortality could be the observation that the decrease in numbers of larvae always occurred after the development of the intestinal loops. We could observe the grazing of algae, but we never observed larvae feeding on the ground fish food applied to stones, as described by Poole in *A. zeteki* tadpoles (2006). Interestingly, she also mentioned that tadpoles stopped feeding at suboptimal temperatures.

It is also possible that there are particular species of algae occurring in the natural habitat, which would have to be provided to successfully rear the tadpoles. We do not exactly know what Harlequin toad larvae feed on (Lötters 1996). Apart from ingesting visible algae, they may also feed on diatoms or bacteria. The density of these organisms may decrease with higher temperatures. Further research is urgently required to answer these questions.

The cause of the malformed legs in the only froglet can also not be explained at this time. The underdevelopment of the forelimbs (arthrogryposis), which is also known as "matchstick legs" or "spindly leg syndrome" (SLS), is a common malformation in anurans and is manifest in thin and stiff forelegs with underdeveloped musculature. In some cases, one or both forelimbs can be completely missing. Affected froglets do not feed and die of starvation after a short time. Causes of the disease have not yet been determined, though genetic factors as well as environmental factors like water temperature, pH value, or malnourishment of tadpoles or parents have been suggested (Köhler 1996). Regarding the high tadpole loss rate after development of the intestinal tract, we cannot exclude the possibility that our larvae were undernourished, although most studies, which regard the disease as diet-related, suggest that insufficient nutrition of the parents and not of the tadpoles (e.g., Heselhaus 1983; Glaw 1987; Krintler 1988) may play a role. Thus, as a consequence, captive bred amphibians in many cases do not seem to be ecologically and physiologically equivalent to offspring from natural populations in the wild.

Concerning individual recognition based on color pattern, we were able to document that the individual pattern remains constant (even if the color of the pattern may change slightly over time); whether this change in color is due to age or environmental factors such as food deserves further study. Because color patterns remain stable, individual photography can be used as a reliable individual recognition method. The advantage of such a method is that it is non-invasive and applicable in the field to various amphibian species (e.g., Kopp-Hamberger 1998; Beukema 2011). We have successfully used this method in an *A. flavescens* population at Noragues, French Guiana (authors' data not shown). Finally, concerning a potential sexual dimorphism in color pattern, further research is required to confirm our preliminary observations.

Outlook

In summary, the seasonal alternation of dry and wet phases appears to be important for successful reproduction of *A. flavescens*. Another relevant factor for the initiation of reproductive activity may be the initial separation of the sexes. A separate terrarium for egg deposition also seems to be advantageous. However, many unanswered questions regarding the successful rearing of *Atelopus* tadpoles still remain.

We recommend a clearly arranged aquatic part of the terrarium for detecting any decrease in tadpole numbers in time, and the placing of appropriate measures for its prevention such as tadpole relocation. We also recommend removing the tadpoles from the adult terrarium and providing them with adequate water amount, under constant control of water conditions and oxygen-content. To ensure sufficient nutrition, algae cultivation should be started ahead of time.

While there are still aspects related to larval rearing that need to be worked out, Cologne Zoo is the only cooperating institute that has so far succeeded in stimulating oviposition and larval development of *A. flavescens*. This highlights the difficulties faced by conservation breeding programs and the necessity of research to evaluate the optimum conditions for reproduction. It is therefore even more important that as many amphibian keeping institutions as possible engage in such programs and research and then subsequently publish their results, because only those experiences will enable the successful, sustainable, and long-term breeding of amphibians in captivity (see also McGregor Reid and Zippel 2008; Ziegler et al. 2011). Finally, husbandry management must not be regarded separately, but should be ideally combined with field research to achieve optimum basic data for successful *ex situ* conservation breeding (e.g., Luger et al. 2009; Lötters et al. 2011).

Acknowledgments.—We would like to thank Ron Gagliardo (Atlanta Botanical Garden) for initiation of the project and the first *Atelopus* transfer. Both our *in situ* and *ex situ Atelopus flavescens* projects were supported by Stiftung Artenschutz/VDZ (Verband deutscher Zoodirektoren e.V.), the European Association of Zoos and Aquaria (EAZA), and the "Centre national de la Recherche Scientifique" (CNRS, French Guiana). Thanks go also to the respective agencies for granting permissions. We also would like to thank Alana Hoenig (Cologne), Martina Luger (Vienna University), Dr. Dennis Rödder (ZFMK), and Philine Werner (Trier University) for their support, and Hannah Lueg (Wuppertal), who improved the English of the manuscript.

Literature cited

Alonso LE, Mol JH. 2007. A rapid biological assessment of the Lely and Nassau Plateaus, Suriname (with additional information on the Brownsberg Plateau). *RAP Bulletin of Biological Assessment* 43:1-276. [Online]. Available: http://www.conservation.org/Documents/RAP_Reports/RAP43_Lely-Nassau_Suriname_Oct-2005.pdf [Accessed: 19 July 2012].

Altig R, McDiarmid RW. 1999. *Tadpoles: The Biology of Anuran Larvae*. The University of Chicago Press, Chicago, Illinois, USA and London, United Kingdom. 458 p.

Beukema W. 2011. Ontogenetic pattern change in amphibians: The case of *Salamandra corsica*. *Acta Herpetologica* 6(2):169-174.

Boistel R, Grosjean S, Lötters S. 2005. Tadpole of *Atelopus franciscus* from French Guyana, with comments on other larvae of the genus and their impact on systematics (Anura: Bufonidae). *Journal of Herpetology* 39(1):148-153.

Bonaccorso E, Guayasamin JM, Méndez D, Speare R. 2003. Chytridiomycosis as a possible cause of population declines in *Atelopus cruciger* (Anura: Bufonidae). *Herpetological Review* 34(4):331-334.

Browne RK, Wolfram K, García G, Bagaturov MF, Pereboom ZJJM. 2011. Zoo-based amphibian research and conservation breeding programs. *Amphibian and Reptile Conservation* 5(3):1-14.

Coloma LA, Lötters S. 1996. The tadpole of *Atelopus balios* (Anura: Bufonidae) from the Pacific lowlands of Ecuador. *Herpetologica* 52(1):66-70.

De la Riva I, Castroviejo-Fisher S, Chaparro JC, Boistel R, Padial JM. 2011. A new species of *Atelopus* (Anura: Bufonidae) from the Amazonian slopes of the Andes in south-eastern Peru. *Salamandra* 47(3):161-168.

Frost DR. 2011. Amphibian Species of the World: An Online Reference. Version 5.5 (31 January 2011). American Museum of Natural History, New York, New York, USA. [Online]. Electronic Database available: http://research.amnh.org/herpetology/amphibia/index.php [Accessed: 28 June 2012].

Glaw F.1987. Probleme mit Streichholzbeinchen bei der Nachzucht von *Discoglossus pictus*. *Herpetofauna* 9(51):30-33.

Gosner KL. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* 16(3):183-190.

Griffiths RA, Pavajeau L. 2008. Captive breeding, reintroduction, and the conservation of amphibians. *Conservation Biology* 22(4):852-861.

Haas W. 1995. Fortpflanzungsbiologie von *Atelopus spumarius spumarius*. *Elaphe N.F.* 3:2-6.

Heselhaus R. 1983. Zum Problem der "Streichholzvorderbeine" bei Dendrobatidennachzuchten. *Herpetofauna* 5(26):22-24.

Heselhaus R. 1994. Erste Nachzucht von *Atelopus varius glyphus*. *DATZ* 47:304-307.

Karraker NE, Richards CL, Ross HL. 2006. Reproductive ecology of the Panamanian golden frog (*Atelopus zeteki*) and comparisons to other members of the genus. *Herpetological Review* 37(3):284-288.

Köhler G. 1996. *Krankheiten der Amphibien und Reptilien*. DATZ Terrarienbücher, Eugen Ulmer Verlag, Stuttgart, Germany. 166 p.

Kok PJR. 2000. A survey of the anuran fauna of Montagne Belvédère, county of Saül, French Guiana: Field list with comments on taxonomy and ecology. *British Herpetological Society Bulletin* 71:6-26.

Kopp-Hamberger M. 1998. Eine Methode zur individuellen Erkennung von Feuersalamandern (*Salamandra salamandra terrestris*) anhand des Zeichnungsmusters. *Salamandra* 34(3):239-244.

Krintler K. 1988. Beobachtungen zum Problem der "Streichholzbeine" bei Dendrobatiden und Hyliden. *Herpetofauna* 10(52):30-31.

La Marca E, Lips KR, Lötters S, Puschendorf R, Ibáñez R, Rueda-Almonacid JV, Schulte R, Marty C, Castro F, Manzanilla-Puppo J, García-Perez JE, Bolaños

F, Chaves G, Pounds JA, Toral E, Young BE. 2005. Catastrophic declines and extinctions in neotropical harlequin frogs (Bufonidae: *Atelopus*). *Biotropica* 37(2):190-201.

Lescure J. 1981. Reference à l'étude des amphibiens de Guyane française IX. Le têtard gastromyzophore d'*Atelopus flavescens* DUMÉRIL et BIBRON (Anura, Bufonidae). *Amphibia-Reptilia* 2(3):209-215.

Lindquist ED, Hetherington TE. 1998. Tadpoles and juveniles of the Panamanian golden frog, *Atelopus zeteki* (Bufonidae), with information on development of coloration and patterning. *Herpetologica* 54(3):370-376.

Lötters S. 1996. *The Neotropical toad genus Atelopus: Checklist, biology, distribution*. M. Vences & F. Glaw Verlags, GbR, Köln, Germany. 143 p.

Lötters S. 2007. The fate of the harlequin toads—help through a synchronous multidisciplinary approach and the IUCN 'Amphibian Conservation Action Plan'? *Zoosystematics and Evolution* 83 (S1):69-73.

Lötters S, Ellwein D, Karbe D, van der Straeten K, Luger M, Rautenberg J, Rödder D, Venegas PJ, Werner P, Ziegler T. 2011a. Erforschung und Schutz der letzten Harlekinfrösche. *Elaphe* 19(2):6-11.

Lötters S, Kielgast J, Bielby J, Schmidtlein S, Bosch J, Veith M, Walker S, Fisher MC, Rödder D. 2010 (2009). The link between rapid enigmatic amphibian decline and the globally emerging chytrid fungus. *EcoHealth* 6(3):358-372.

Lötters S, van der Meijden A, Coloma LA, Boistel R, Cloetens P, Ernst R, Lehr E, Veith M. 2011. Assessing the molecular phylogeny of a near extinct group of vertebrates: The Neotropical harlequin frogs (Bufonidae; *Atelopus*). *Systematics and Biodiversity* 9(1):45-57.

Luger M, Hödl W, Lötters S. 2009. Site fidelity, home range behaviour and habit utilization of male harlequin toads (Amphibia: *Atelopus hoogmoedi*) from Suriname: Relevant aspects for conservation breeding. *Salamandra* 45(4):211-218.

Lynch JD. 1986. Notes on the reproductive biology of *Atelopus subornatus*. *Journal of Herpetology* 20(1):126-129.

McGregor Reid G, Zippel KC. 2008. Can zoos and aquariums ensure the survival of amphibians in the 21st century? *International Zoo Yearbook* 42(1):1-6.

Mebs D. 1980. Zur Fortpflanzung von *Atelopus cruciger* (Amphibia: Salientia: Bufonidae). *Salamandra* 16(2):65-81.

Poole V. 2006. *Panamanian Golden Frog Husbandry Manual*. Second edition. [Online]. Available: http://www.ranadorada.org/species-info.html [Accessed: 08 March 2012].

Pounds JA, Bustamante MR, Coloma LA, Consuegra JA, Fogden MPL, Foster PN, La Marca E, Masters KL, Merino-Viteri A, Puschendorf R, Ron SR, Sánchez-Azofeifa GA, Still CJ, Young BE. 2006. Widespread amphibian extinctions from epidemic disease driven by global warming. *Nature* 439(7073):161-167.

Rueda-Almonacid JV, Rodríguez-Mahecha JV, La Marca E, Lötters S, Kahn T, Angulo A (Editors). 2005. *Ranas arlequines*. Serie Libretas de Campo 5. Conservación Internacional Colombia, Bogotá DE (Colombia). 158 p.

Siavichay F, Alvarado D, Arbeláez Ortiz E, y Martínez R, CC. 2011. Amplexus, egg deposition and tadpole hatching in the Black Cajas harlequin toad at the Amphibian Conservation Center - Mazán forest in Ecuador. *Newsletter Amphibian Ark* (AARK Newsletter) 15. [Online]. Available: http://www.amphibianark.org/Newsletters/amphibian_ark_news_15.pdf [Accessed: 18 July 2012].

Stuart SN, Hoffman M, Chanson J, Cox N, Berridge R, Ramani P, Young B. 2008. *Threatened Amphibians of the World*. Lynx editions, Barcelona, Spain; IUCN, Gland, Switzerland; Conservation International, Arlington, Virginia, USA. 776 p.

Ziegler T, Dang TT, Nguyen TQ. 2011. Breeding, natural history and diversity research: *Ex situ* and *in situ* Asian amphibian projects of the Cologne Zoo and the Institute of Ecology and Biological Resources. In: *Biology and Conservation of Tropical Asian Amphibians.* Editors, I Das, A Haas, AA Tuen. Proceedings of the Conference "Biology of the amphibians in the Sunda region, South-East Asia," Sarawak, Malaysia, 28-30 September 2009. Institute of Biodiversity and Environmental Conservation, Universiti Malaysia Sarawak, Kota Samarahan. 137-146.

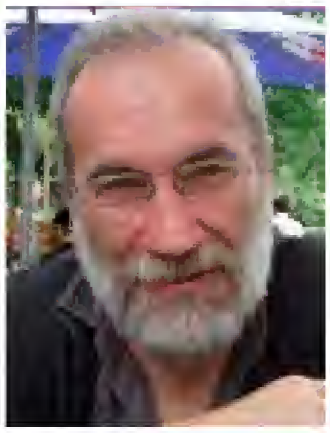
Zippel K, Johnson K, Gagliardo R, Gibson R, McFadden R, Browne R, Martinez C, Townsend E. 2011. The amphibian ark: A global community for *ex situ* conservation of amphibians. *Herpetological Conservation and Biology* 6(3):340-352.

Received: 28 April 2012
Accepted: 08 July 2012
Published: 31 August 2012

Note added in proof: While the present paper was in press, the *Atelopus* taxon dealt with in this article was described as new subspecies *Atelopus hoogmoedi nassaui* by: Ouboter PE, Jairam, R. 2012. *Fauna of Suriname. Amphibians of Suriname.* Brill, Leiden. 376 p.

Anna Gawor completed her master thesis at the Faculty of Mathematics and Natural Sciences at the University of Bonn in collaboration with the Cologne Zoo and the Institute of Ecology and Biological Resources in Hanoi, Vietnam in 2011, focusing on the biodiversity of the herpetofauna of the Bai Tu Long National Park. Since 2007, she has been involved in various projects at the Cologne Zoo dealing with tropical batrachology, resulting so far in five scientific publications. Her interests comprise systematics, ecology, and diversity of amphibians, in particular reproduction of anurans, monitoring of larval development as well as larval morphology.

Anna Rauhaus started her career at the Aquarium/Terrarium Department of the Cologne Zoo in May 2011. She finished her apprenticeship as zoo keeper in the year 2010. Her focus of expertise is herpetology and behavioral training.



Detlef Karbe has been employed at the Cologne Zoo since 1974. He has worked for 20 years as a gardener in the Aquarium/Terrarium Department and then continued on as a zoo keeper. His main focus of work is the construction of amphibian facilities and the husbandry and breeding of anurans and salamanders.

Karin van der Straeten has been employed at the Cologne Zoo since 1970. She is a zoo keeper with a focus on amphibians and reptiles. During her career she has successfully bred more than ten species of amphibians. She is head keeper in the Terrarium Department.

Stefan Lötters is an assistant professor at Trier University with focal research on ecology, evolution, and systematics of amphibians from the Amazon and Congo basins. Harlequin toads are among his key groups. Before, he did research postdocs at the universities of Amsterdam and Mainz where he started to engagement in amphibian conservation projects. Stefan has also contributed to the global *IUCN Amphibian Conservation Action Plan*.

Thomas Ziegler has been the curator of the Aquarium/Terrarium Department of the Cologne Zoo since 2003. He completed his herpetological Ph.D. in the year 2000 at the Rhineland Friedrich Wilhelms University Bonn. Thomas so far has conducted herpetological field work in South America (Paraguay) and South East Asia (Vietnam, Laos). Since 1994, he has published 252 papers and books, mainly dealing with herpetodiversity. His main research interests include diversity, systematics, and zoo biology of amphibians, geckos, monitor lizards, snakes, and crocodiles. Since February 2009, he has been an associate professor at the Zoological Institute (Biocentre) of Cologne University.

Copyright: © 2012 Preininger et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Amphibian & Reptile Conservation 5(3):45-56.

The conservation breeding of two foot-flagging frog species from Borneo, *Staurois parvus* and *Staurois guttatus*

[1,3]Doris Preininger, [2]Anton Weissenbacher, [2]Thomas Wampula, and [1]Walter Hödl

[1]*Department of Evolutionary Biology, University of Vienna, Althanstraße 14 A-1090 Vienna, AUSTRIA*
[2]*Vienna Zoo, Maxingstraße 13B A-1130 Vienna, AUSTRIA*

***Abstract.*—The Bornean frogs of the genus *Staurois* live exclusively along fast-flowing, clear water rainforest streams, and are famous for displaying a variety of visual signals, including foot flagging. Their extraordinary behavior, and the continued loss of their natural habitat due to deforestation and subsequent pollution, make them a group of target species for captive breeding, as well as behavioral research. The Vienna Zoo has pioneered in the development of a research and conservation project for *S. parvus* and *S. guttatus*. We implemented two breeding and research arenas, offering an artificial waterfall and different options for egg deposition in a bio-secure container facility. Two months after introducing the frogs, we observed amplectant pairs and the first tadpoles of *S. parvus* and *S. guttatus*. The Vienna Zoo is the first zoo worldwide that has succeeded in breeding foot-flagging frog species and meanwhile has recorded over 900 tadpoles and at least 470 juveniles. One of the most striking observations has been the use of foot-flagging signals in recently metamorphosed *S. parvus*. This corroborates our assumption that "foot flagging" is employed as intraspecific spacing mechanism. The breeding success of two *Staurois* species at the Vienna Zoo can help in species conservation as it increases our knowledge on conditions necessary to breed tropical stream-dwelling anuran species found to be particularly threatened in nature. Furthermore, the captive colony provides research conditions to better understand the role of "foot flagging" as a visual signal component in anuran communication.**

Key words. Amphibia, anura, bio-secure management, conservation research, *ex situ* breeding

Citation: Preininger D, Weissenbacher A, Wampula T, Hödl W. 2012. The conservation breeding of two foot-flagging frog species from Borneo, *Staurois parvus* and *Staurois guttatus*. *Amphibian & Reptile Conservation* 5(3):45-56(e51).

Introduction

Amphibian species are declining in many parts of the world. On average 41% of amphibians are classified as Threatened on the International Union of Conservation of Nature (IUCN) Red List. The extinction risk in South East Asia still increases (Hoffmann et al. 2010). Only recently an Amphibian Conservation Action Plan has been developed, which states important priorities for relevant amphibian research and conservation. Understanding the cause of decline, assessing changing diversity and implementing long-term conservation programs are some of the immediate interventions necessary to conserve amphibians (Gascon et al. 2007). Zoo-based amphibian research and conservation breeding programs facilitating *ex situ* and *in situ* conservation of amphibian species have been established for a wide range of species over the last decades (Browne et al. 2011; Gagliardo et al. 2008; Lee et al. 2006; McFadden et al. 2008).

In South East Asia, habitat loss and destruction is one of the main causes for the rapid decline of amphibian species (Stuart et al. 2004). Deforestation of natural habitats increases siltation and chemical pollution in streams. Few stream-dwelling Bornean species are able to survive in habitats modified for human use (Inger and Stuebing 2005). A recent study carried out in Brunei demonstrated that deforestation due to road construction enabled *Limnonectes ingeri* to migrate more than 500 m into primary forest, which posed a potential threat to native amphibian assemblages (Konopik 2010). Inger and Stuebing (2005) mentioned an increase of the Giant river frog (*Limnonectes leporinus*) along silted streams of logged areas and a simultaneous decrease in some species of Torrent frogs (*Meristogenys* spp.). About half the frog species in Southeast Asia are restricted to riparian habitats and develop in streams (Inger 1969; Zimmerman and Simberloff 1996). Most anuran stream-side communities in Borneo are known to breed in clear, turbulent water and are absent in streams with silt bottoms that are lacking riffles and torrents (Inger and Voris 1993). The heterogeneity of riparian habitats in pristine rainforests results in reoccurring stream assemblages and habitat specific adaptations (Keller et al. 2009).

Correspondence. *Email:* [3]*doris.preininger@univie.ac.at*

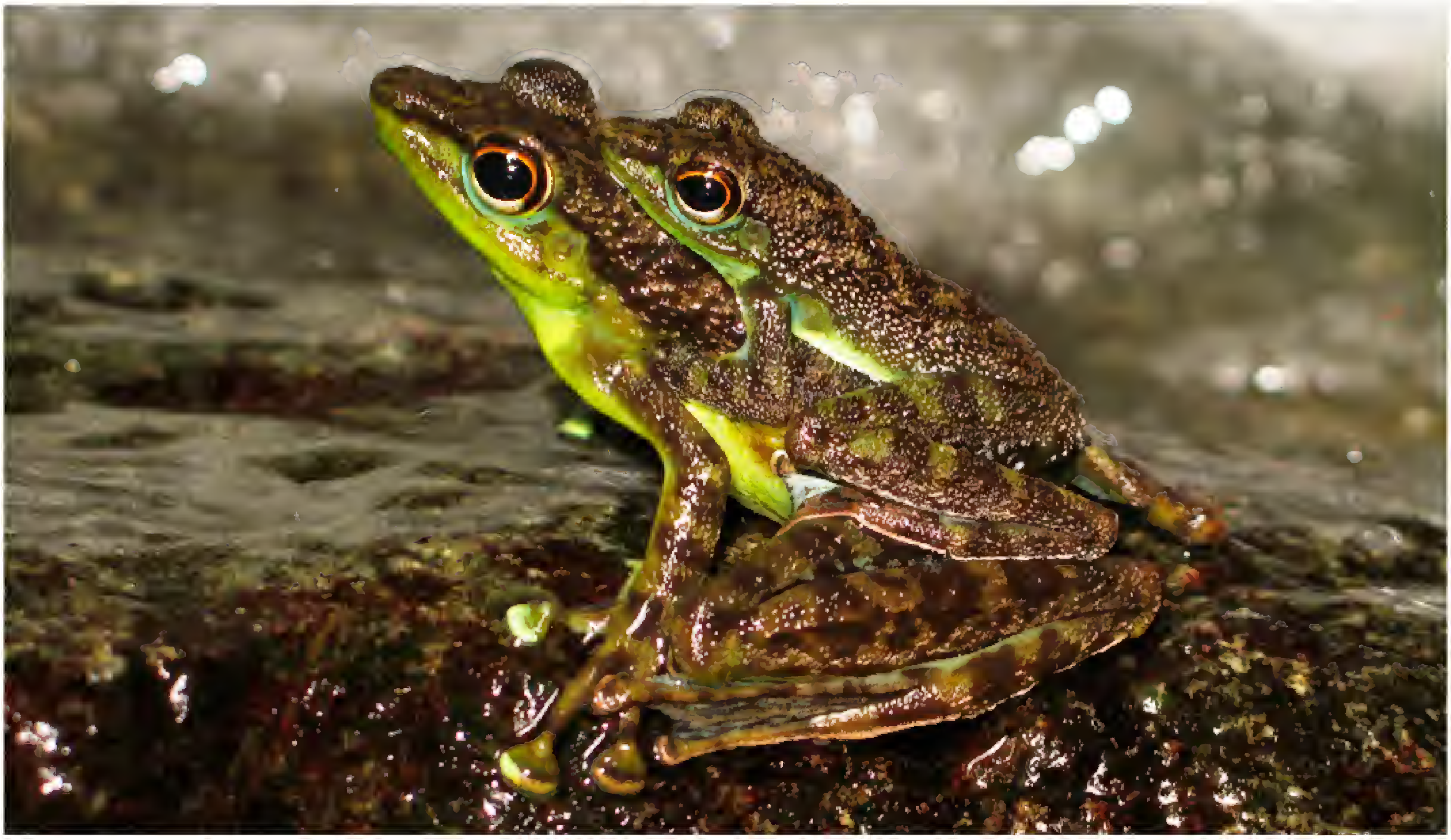


Figure 1. Male and female *Staurois guttatus* in amplexus resting at a waterfall. *Image by M. Böckle.*

Many stream living anuran species in Borneo show morphological and behavioral adaptations to torrential streams and waterfalls. For example, the tadpoles of *Huia cavitympanum* and of all species of the genus *Meristogenys* have large abdominal suckers specialized for a life in currents (Haas and Das 2012). The adult males of *M. orphnocnemis* use high frequency calls to communicate in noisy stream environments (Boeckle et al. 2009; Preininger et al. 2007). An extraordinary spectral adaptation to enhance the signal-to-noise ratio has also been reported in *Huia cavitympanum*, in which males call in a band of ultrasonic frequencies (Arch et al. 2008). In the vicinity of waterfalls and fast-flowing streams, species of the genus *Staurois* display an exceptional behavior termed, "foot-flagging" (Grafe et al. 2012; Grafe and Wanger 2007; Preininger et al. 2009). The conspicuous visual display mainly observed in tropical anuran species inhabiting riparian habitats (reviewed in Hödl and Amézquita 2001) may act as a complementary mode of communication in noisy habitats.

The Bornean foot-flagging species, *Staurois guttatus* (Fig. 1) and *S. parvus* (Fig. 2) occur in sympatry, but use different microhabitats along streams. Both species have solved the problem of continuous broadband low-frequency noise by modifying their advertisement calls to increase in pitch and use numerous visual signals (Grafe et al. 2012; Grafe and Wanger 2007). Males of *S. guttatus* perch on vegetation along fast flowing streams and waterfalls. Individuals of *S. parvus* display along steep rock formations close to the waterline (D. Preininger, pers. observ.). The breeding behavior and habitat of tadpoles are unknown from *S. parvus*, though given the microhabitats of the adults tadpoles probably live in currents along the stream. *Staurois guttatus* tadpoles, however, have been found in leaf litter in side pools of streams (Haas and Das 2012) similar to an unidentified Bornean tadpole of a ranid genus with slender body shape and nearly pigmentless skin resembling neotropical centrolenid larvae (Inger and Wassersug 1990). *Staurois parvus* has recently been resurrected from the synonym with *S. tuberilinguis* (Arifin et al. 2011; Matsui et al. 2007). The tadpoles of *S. tuberilinguis*, reported by Malkmus et al. (1999), exhibit a fossorial life in leaf litter at the margins of forest streams. The IUCN Red List categorizes *S. tuberilinguis* as "Near Threatened" with a decreasing population trend (Inger et al. 2004), and *S. parvus* and *S. guttatus* are listed as "Data Deficient" (IUCN 2011).

In 2008, in light of the "Year of the Frog" campaign initiated by the World Association of Zoos and Aquariums (WAZA) and the IUCN we started a unique conservation and research project. A bio-secure container facility was constructed and with permission of the Universiti of Brunei Darussalam and the Brunei Museums Department we imported ten individuals of *S. guttatus* and ten individuals of *S. parvus* to the Vienna Zoo. Apart from several research aspects concerning the remarkable multimodal (visual and acoustic) signals employed in communication, we were especially interested in the reproductive behavior and the accompanying conditions crucial for reproductive success. We here report our first findings in *ex situ* management and breeding of *S. parvus* and *S. guttatus*.

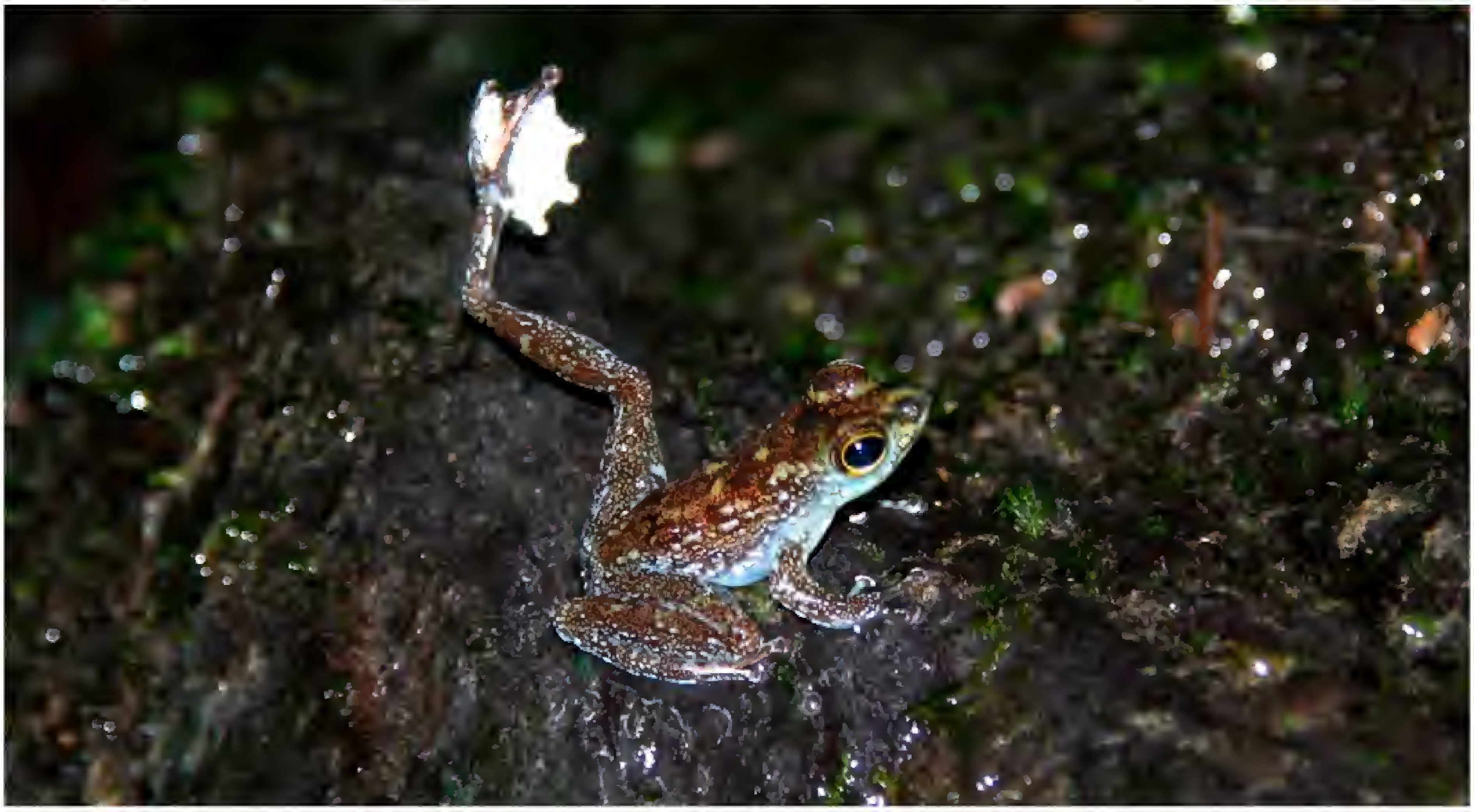


Figure 2. A male of *Staurois parvus* displaying the white interdigital webbing during foot-flagging behavior. The visual signals are mainly employed during male-male agonistic interactions. *Image by D. Preininger.*

Methods

Study species

In May 2010 we collected 20 individuals (ten pairs) of the species *S. parvus* and *S. guttatus* in the Ulu Temburong National Park, Brunei Darussalam, Borneo. Frogs were located at narrow, rocky (black shale) sections of the Sg. Anak Apan and Sg. Mata Ikan (Fig. 3), two small freshwater streams that merge into the Belalong River close to the Kuala Belalong Field Studies Centre (115°09´E, 4°33´N). *Staurois parvus* is a ranid frog, endemic to Borneo. Males are diurnal and perch on rocks along fast-flowing forest streams. Their white chest and webbing between the toes of the hind legs strongly contrast to their cryptic dark grey, brown dorsal body. The snout-urostyle length and weight of the investigated population of male *S. parvus* averaged 21.5 ± 0.5 mm (*n* = 13) and 0.7 ± 0.05 g (*n* = 13) (Grafe et al. 2012) and of females 29.5 ± 1.8 mm (*n* = 5) and 1.7 ± 0.2 g (*n* = 5) (Preininger et al., data not shown). The closely related species *S. guttatus* occurs throughout Borneo. It was previously known as *Staurois natator* (Inger and Tan 1996), a name still used for populations in the Philippines (Iskandar and Colijn 2000). Males of this diurnal species perch on rocks and branches along fast-flowing mountain streams. Females were found 10-50 m away from the river under overhanging rock formations and tree branches. The snout-urostyle length and weight ± SE of the investigated population of male *S. guttatus* averaged 33.6 ± 0.4 mm (*n* = 14) and 2.69 ± 0.07 g (*n* = 14), that of females 50.1 ± 0.3 mm (*n* = 6) and 9.74 ± 0.2 g (*n* = 6) (Preininger et al., data not shown).

Individuals were collected with permission of the Brunei Museums Department.

Ex situ breeding facility

In the Vienna Zoo two connected bio-secure containers, fully isolated from other facilities were implemented as the research complex for the animals (Fig. 4). The use of converted shipping containers for the *ex situ* breeding and management of amphibians was pioneered by Gerry Marantelli at the Amphibian Research Centre (ARC) in Melbourne, Australia. The Vienna Zoo has tested specimen (including *S. parvus* and *S. guttatus*) for infection with the chytrid fungus and no positives were detected. At the start of the project we kept individuals in pairs in medium sized terraria (50 × 60 × 70 cm) in the container facility that contained some tree branches, plants, stones, and flowing water which ran over potsherd. We also built a research arena (150 × 120 × 100 cm) for behavioral experiments that we converted into a breeding arena in 2011 (Fig. 5) to improve space requirements because neither of the species had reproduced in their original terraria. We implemented a controllable waterfall with several smaller cascades creating areas of flowing and dripping water that additionally increased humidity levels. Small burrows, ledges, and perching sites were built out of foamed polystyrene. Similar to the smaller terrariums we added plants with large leaves (*Monstera* sp., *Philodendron* sp., *Spathiphyllum* sp., *Dieffenbachia* sp.,

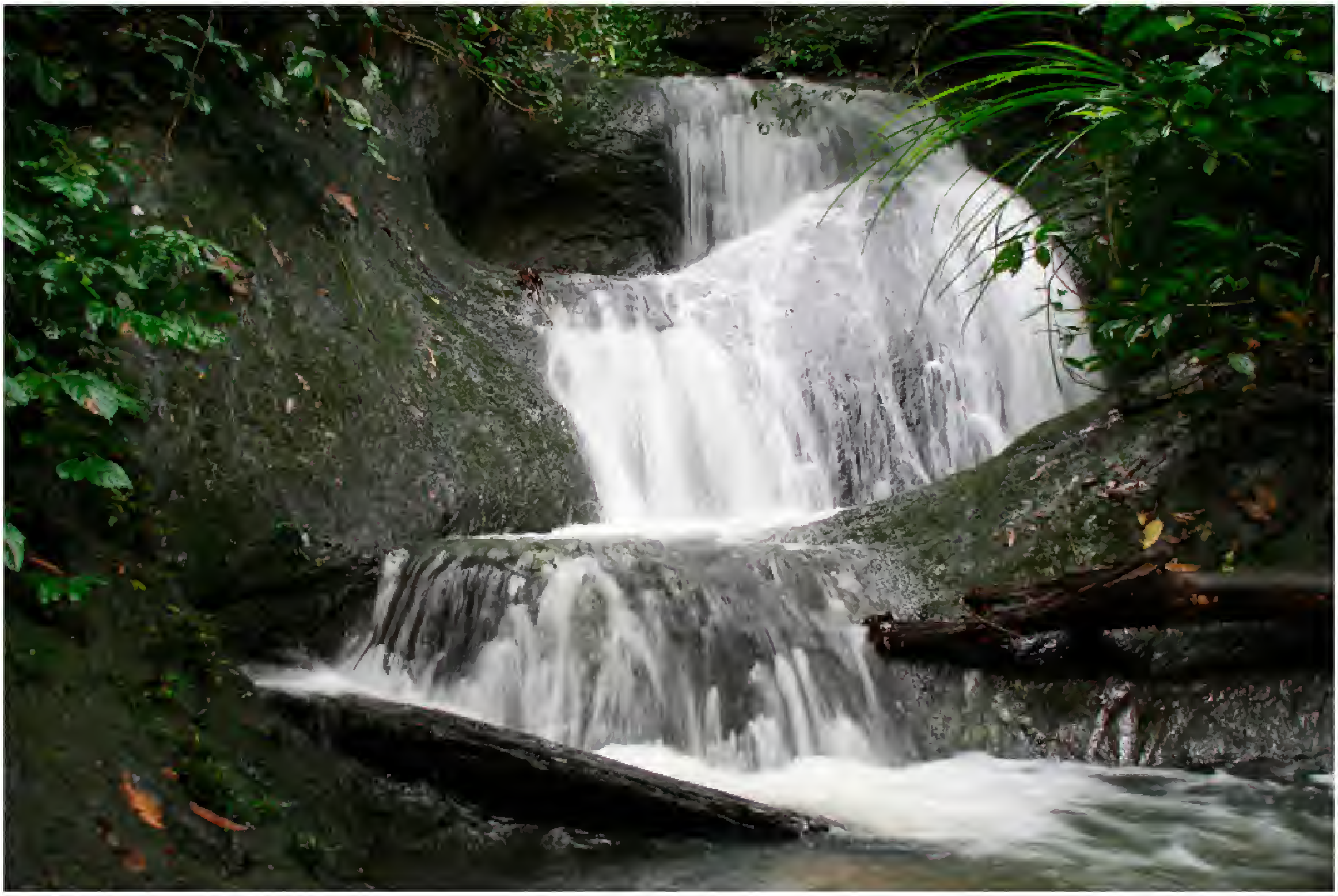


Figure 3. A waterfall habiat of *Staurois guttatus* at the Sungai Mata Ikan ("Fish-Eye" River) in the Temborong District in Brunei, Borneo. *Image by D. Preininger.*

Aglaonema sp., *Scindapsus* sp., and others) as nightly resting sites. We incorporated a self-built rain and misting system to simulate rainy and dry periods. The water area, which covered the entire floor of the terrarium, was filled with gravel of different grain sizes and larger pebbles that provided perching sites and interstitial spaces. We further installed two smaller glass containers (30 × 30 × 30 cm), one placed directly under the waterfall mimicking a constantly flushed pool with large stones, and the other containing sand, dead leaves, and standing water, as found in side ponds of waterfalls. A mixture of osmosis-purified water and drinking water (average conductivity = 9 µS/cm, pH = 7.2) was discharged via the waterfall and drained into an external filter reservoir, which created a slow current in the main water area. As light source we used a metal-halide lamp (HIT-DE 70 Watt [Daylight]) and placed several plastic boards on top of the terrarium to mimic canopy coverage. Individuals were housed under 12-hour light, 12-hour dark cycles. We placed five pairs of *S. parvus* into the arena. From then on individuals could only be counted at night when perching on leaves, while frogs rested in the many hiding places during the day.

A similar facility (150 × 150 × 150 cm) was constructed for *S. guttatus*, however the water area did not contain additional artificial pools or ponds, and the waterfall was amended with several tree branches. Temperature in both facilities averaged 25 °C (range: 22-27 °C) and closely resembled the natural habitat temperature (Fig. 6). Relative humidity ranged from 95% to 100%. For a period of 14 days we simulated a dry period with no rain and decreased water levels (10 cm), followed by a 14 day rainy period with four hours daily rainfall (7-8am and 5-8pm), elevated water levels (15 cm) and an increased quantity of water flowing over the waterfall. This procedure was repeated with the intervals between the dry and rainy periods reduced to seven days, and rain periods adjusted to different times of day (e.g., 5-10pm and no morning rain). We also played back conspecific advertisement calls recorded in the field, during peak activity periods (9-11am and 3-5pm).

Adult frogs were fed with gut-loaded House crickets (*Acheta domesticus*), Firebrat (*Thermobia domestica*), and blow flies (*Lucilia* sp.); tadpoles received algae tablets, fish food flakes, and fish filet; the diet of metamorphosed frogs consisted of *Drosophila* sp. and *Collembola*. All feeder insects were dusted with a vitamin and mineral mixture (Vitakalk, Korvimin or Nekton MSA).

Tadpoles were photographed in petri-dishes on graph paper and snout-vent length (SVL) and Gosner stage (Gosner 1960) derived from the photos. We measured SVL and body mass of juvenile *S. parvus* with a sliding caliper to the nearest 0.1 mm, and a digital mini scale to the nearest 0.01 g. Tadpole specimens of various stages of *S. parvus* were deposited at the Austrian Natural History Museum (*Staurois parvus* larvae: NHMW 39337).

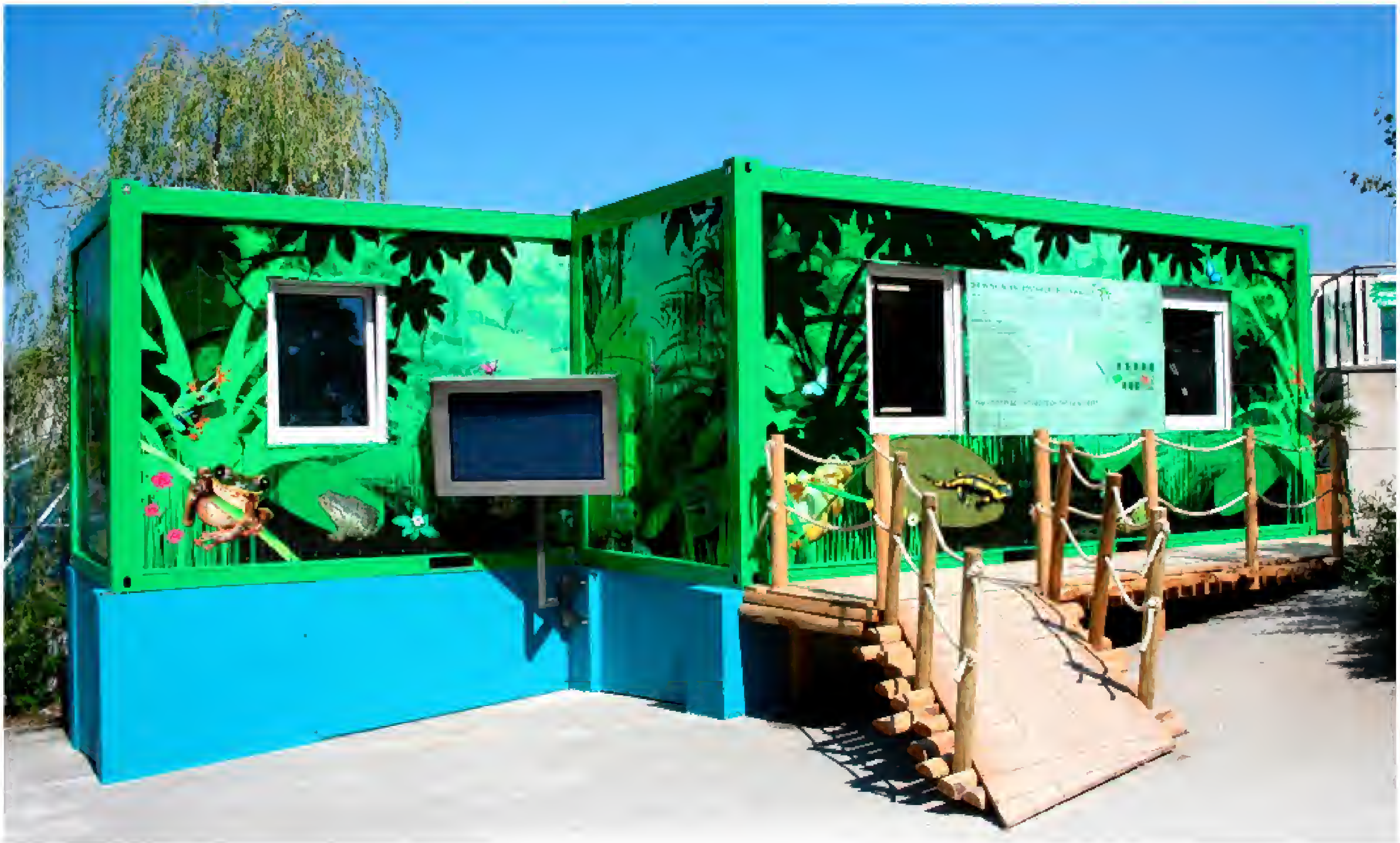


Figure 4. The bio-secure container facility a modern Noah´s Ark, which houses *Staurois guttatus* and *S. parvus* at the Vienna Zoo Schönbrunn. *Image by D. Preininger.*

Results

Staurois parvus

On 18 October 2011 we observed the first three tadpoles of *S. parvus* during an evening census of adult individuals in the gravel of the slow-flowing current area of the terrarium. When a tadpole could first be captured it was in Gosner stage 25 and measured 11.2 mm in total length (SVL: 3.3 mm, *n* = 1) and was completely transparent (Fig. 7). Due to the transparency of the body, the organs and blood vessels shined through the skin and the body was of reddish appearance. The highly photophobe individuals colonized the interstitial spaces of the gravel area. More tadpoles staged 26-28, captured 24 days later, measured ca. 21 mm in total lengths (SVL: 6 mm, *n* = 1) and the body and tail were covered with dorsal black spots. After complete toe development (> stage 38) individuals showed a brown coloration with green iridescence and a yellow iris, as seen in adults. At this stage, 70 days after the first sighting, individual length was 41 mm (SVL: 12 mm, *n* = 1). At the end of metamorphosis the dorsal coloration of individuals turned into bright green (Fig. 8).

The first metamorphosed *S. parvus* left the water on 30 January 2012 (SVL: 13 mm, tail-length: 6 mm), 104 days after we observed the first tadpoles. To date, we house 285 froglets in separate terraria in the bio-secure container, over 600 tadpoles and 180 juveniles have been raised for approximately 30 days and afterwards released at an artificial waterfall in the Rainforest house of the zoo (Fig. 9), where the establishment of a semi-wild population is intended. The metamorphs have dark green or black spots and small tuberculi on the dorsal side, the latter eponymous for the closely related species *S. tuberilinguis*. They measured 11.8 mm (mean SVL, SD ± 0.8, *n* = 20) and had a body mass of 0.12 g (SD ± 0.03, *n* = 20).

Due to the high reproductive success we recently allowed disturbance at the setup in order to search for egg-deposition sites. So far, we have discovered two clutches of eggs that were attached under big stones in the slow-flowing water current. Surprisingly, with respect to the large tadpole numbers in the project, those two clutches contained only 14 and 26 eggs, respectively. The survival rate of 120 separated tadpoles (tank A: *n* = 40, tank B: *n* = 80) was 87% (tank A: *n* = 34, 85%; tank B: *n* = 71, 88.8%). Presently, we house over 200 tadpoles, 6-10 juveniles and nine adults in the breeding facility.

Metamorphosed frogs were placed into separate terraria, only hours after leaving the water, and were immediately observed to display foot-flagging behavior (Fig. 10). The young frogs performed complete foot-flags, in which the leg is raised and the toes are spread as observed in adult individuals. Interdigital webbings were colored transparent grey and did not exhibit the contrasting white coloration as seen in adults.

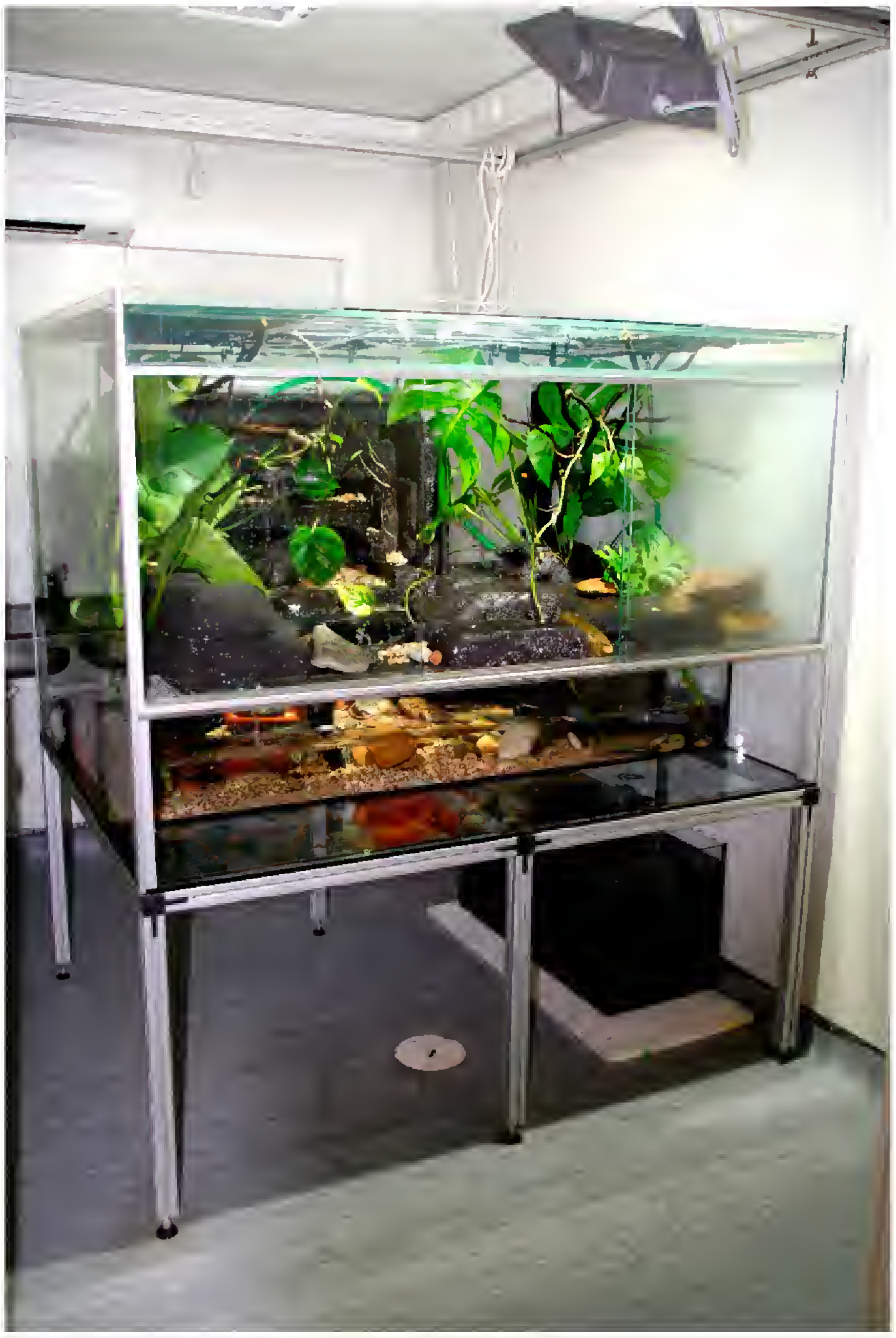


Figure 5. *Ex situ* breeding facility designed to offer different egg deposition sites (described in detail in the Methods section). *Image by D. Preininger.*

Staurois guttatus

The first tadpoles of *S. guttatus* were observed on 20 March 2012, approximately 11 days after observing a pair in amplexus. In the estimated development stage 23-24, 36 days after discovery, the tadpoles had a mean length of 30 mm (8 mm SVL, range: 7-9 mm; 22 mm tail length, range: 21-24 mm, $n = 5$). At this stage, the dorsal body and tail was a light brown color and the body was transparent with a grey iridescence (Fig. 11). A darker dorsal line ran from the top of the head to the tip of the tail and a ventral line could be observed on both sides of the tail. So far we have moved 76 tadpoles to a separate aquarium and approximately 50 are housed in the breeding facility.

Discussion

The combined efforts of members of the Vienna Zoo, University of Vienna, and the Universiti of Brunei Darussalam have established a research and conservation project that succeeded to breed the foot-flagging frogs *Staurois guttatus* and *S. parvus ex situ*. Zoo-based research and conservation breeding programs focusing on amphibians have gained global support and resulted in increased conservation efforts for many threatened species (Browne et al. 2011). Information on natural history, reproduction modes, and behavior of anurans is important to determine and protect key-habitats.

The tadpoles of *S. guttatus* and *S. parvus* colonized the hyporheic interstitial in the slow-flowing current areas in the breeding facility, which supports our assumption that the larvae develop in fresh water streams or adjacent pools of fast-flowing mountain streams and waterfalls. On two occasions we found eggs of *S. parvus* in underwater gaps between larger rocks and the subjacent gravel of our breeding terrarium. Neither in the artificially flushed pool with large pebbles, nor in the sand and leaf filled container mimicking a side pool of the waterfall, tadpoles or eggs could be observed. In a stream-dwelling, foot-flagging species from Brazil (*Hylodes dactylocinus*) males dig underwater chambers prior to courtship and eggs are deposited on the sandy bottom between rocks along streams (Narvaes and Rodrigues 2005). Another diurnal species (Micrixalidae: *Micrixalus saxicola*) displays foot-flagging signals and lives along perennial streams in the Western Ghats, India. Females of *M. saxicola* dig under-water cavities with the hind legs in gravel areas of flowing streams while in amplexus with a male or before courtship (Gururaja 2010; D. Preininger, pers. observ.). Although we did not observe *S. parvus* males or females digging under-water chambers, we assume that sufficient gaps between rocks could provide similar protection from predators. We observed amplectant pairs at the study site in Brunei to repeatedly move up the stream only to dive back into pools at the bottom of cascades and smaller waterfalls over a period of 1-2 days. This behavior could indicate either the search for suitable deposition sites or the deposition of several clutches.

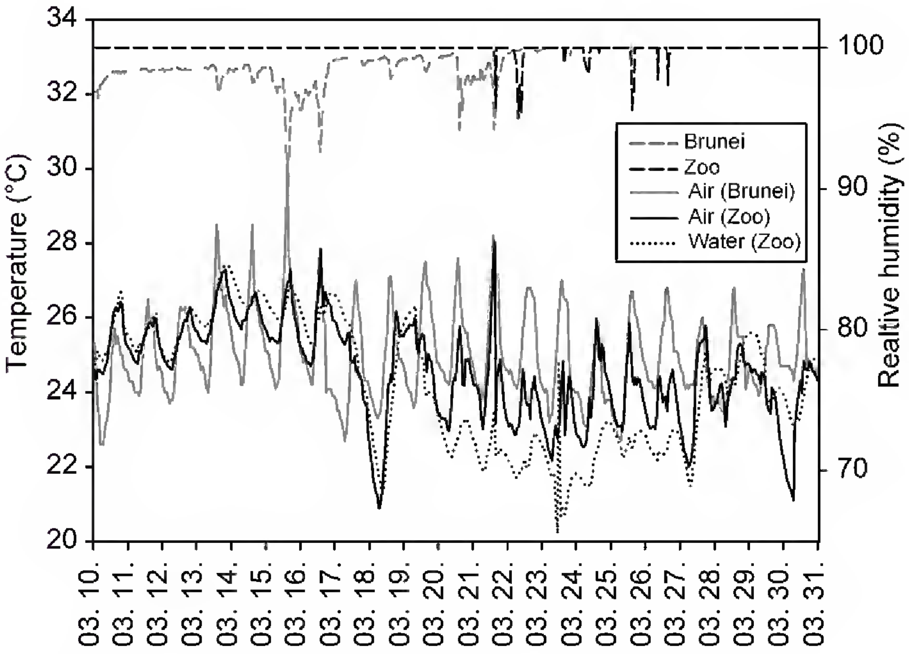


Figure 6. Comparison of temperatures and relative humidity measured for a period of three weeks in the natural habitat in Brunei (2010) and the breeding facility in the Vienna Zoo (2012). Solid lines represent air temperature, dotted line water temperature, and dashed lines denote relative humidity in the respective habitat.

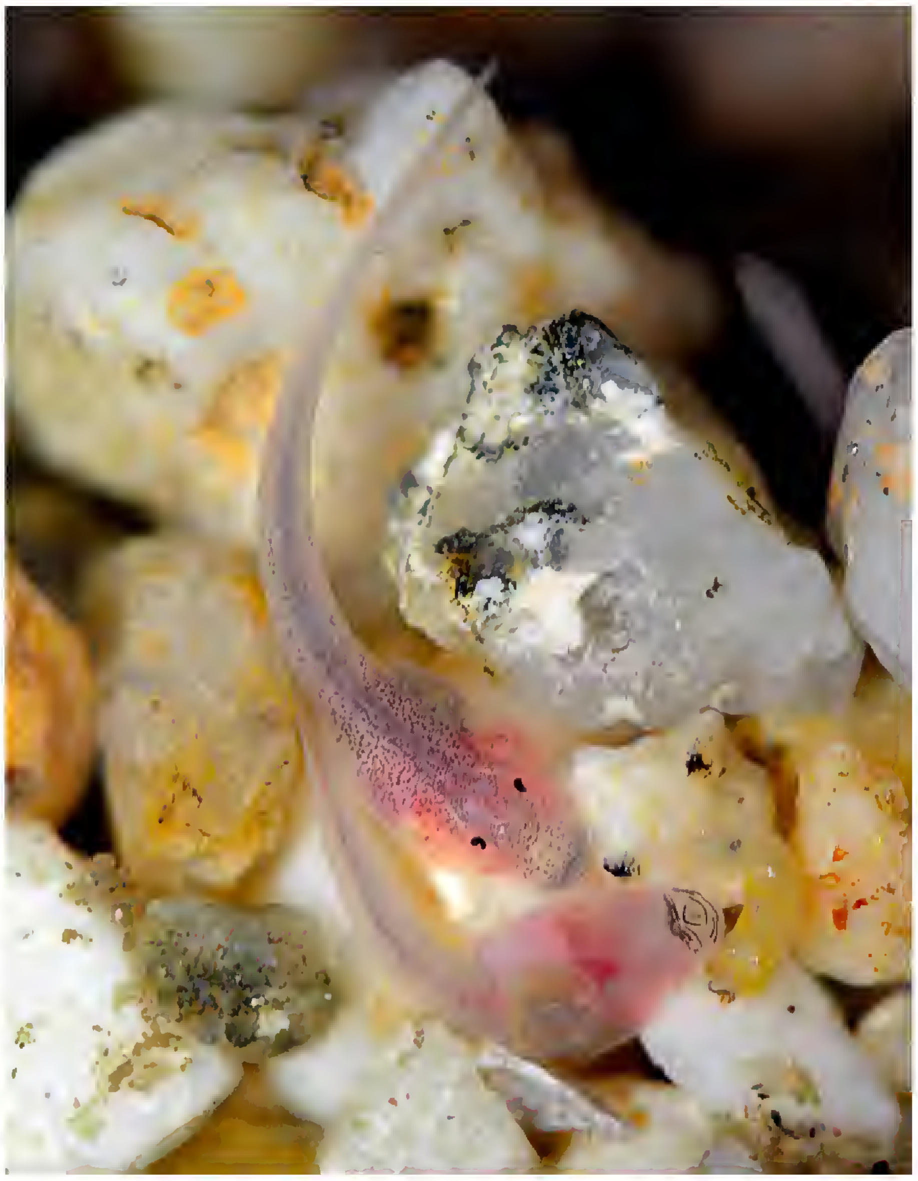


Figure 7. Tadpoles of *Staurois parvus. Image by N. Potensky.*

The diversely structured artificial habitat in the breeding tank offered individuals similar conditions as observed in the natural habitat. Earlier studies that kept adults of *S. parvus* in terrariums of simpler design (no flowing water) showed that individuals did not display acoustic or visual signals under such conditions (R. Kasah, pers. comm.). At the beginning of our project we kept individuals pair-wise in simpler terraria with a small water area containing no gravel and only larger pebbles, some tree branches, flowing water via a pump, and temperatures of 23-25 °C. Under these conditions individuals performed advertisement calls and foot-flagging behavior but no reproductive behavior could be observed. Especially in *S. guttatus* females displayed territorial calls and foot flags if males approached, a behavior that was interpreted as a spacing mechanism (Preininger et al., data not shown). After transferring all individuals in the considerably larger and diversely structured breeding tank, calling activity intensified, and pairs in amplexus could be observed after a few weeks. Hence, we suggest that first and foremost the gravel containing flowing water area was crucial for reproduction, but also the simulated dry and rainy season might have had an effect. It is now essential to alter or exclude single environmental conditions or habitat structures to determine factors necessary for reproduction. So far we have removed the artificial side pool and flushed

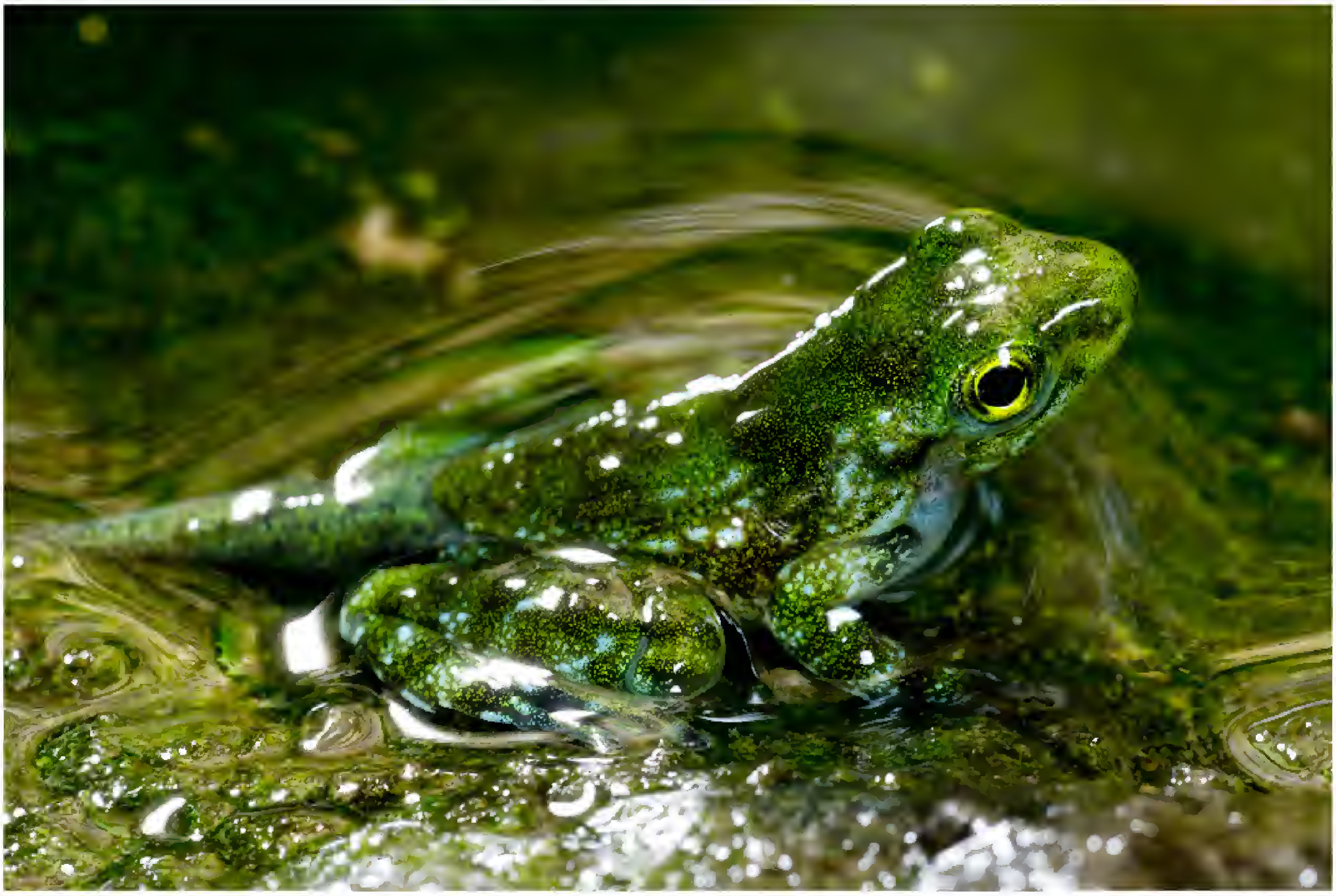


Figure 8. Juvenile *Staurois parvus. Image by D. Zupanc.*

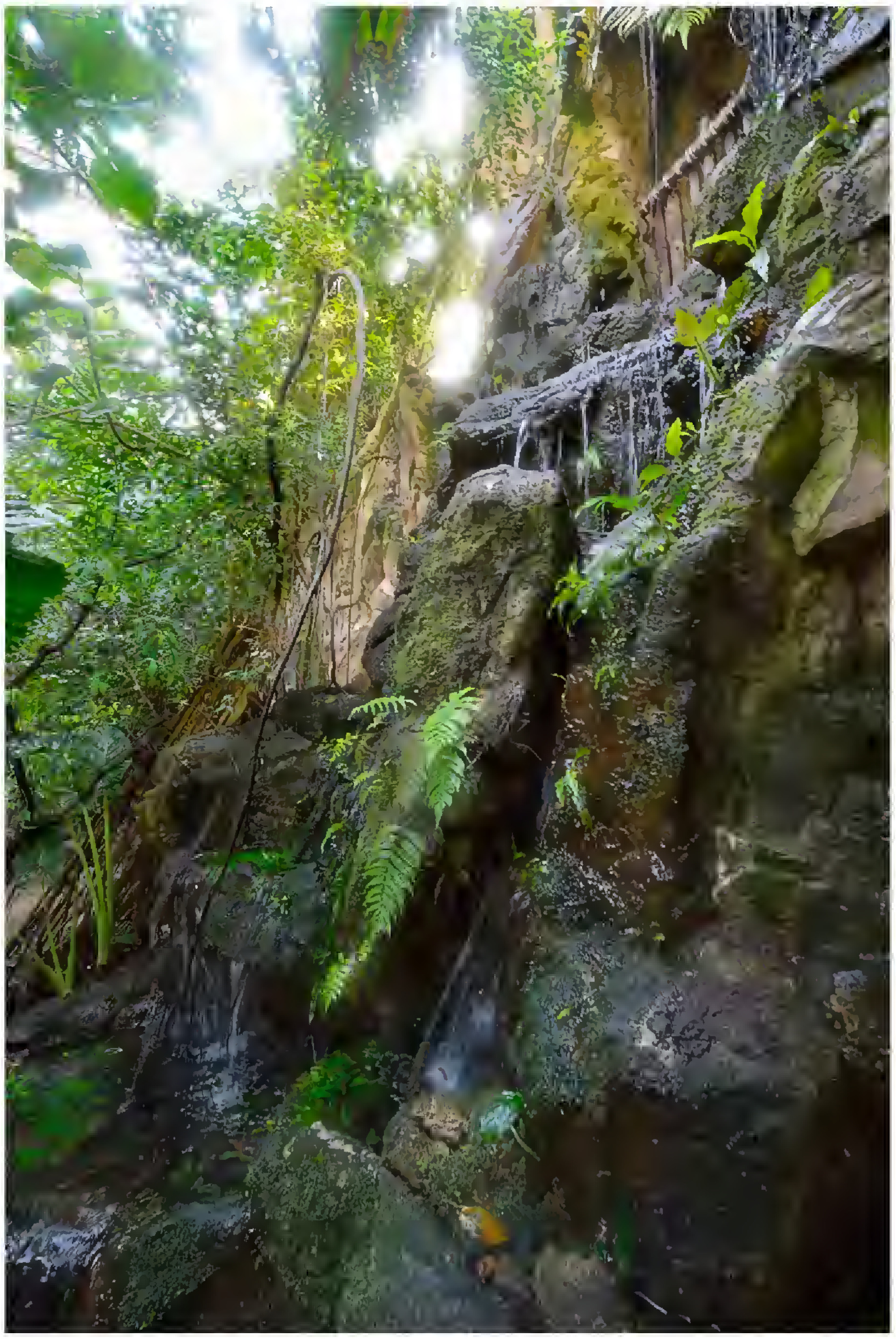


Figure 9. Artificial waterfall habitat at the Borneo Rainforest-house in the Vienna Zoo. *Image by N. Potensky.*

pool from the *S. parvus* breeding terrarium and still observe freshly hatched tadpoles.

Freshwater streams and adjacent flown-through pools with gravel areas seem to be important to secure the survival of the foot-flagging species in the genus *Staurois*. However, deforestation and subsequent siltation of streams and rivers are the major threats to most stream-living and breeding anuran species in Borneo. Inger and Voris (1993) found that a stream with a silt bottom completely lacked all the species known to breed along clear and fast-flowing streams. Selective logging changes the water chemistry considerably in nearby streams and sediment yields of streams are 18 times higher for up to five months after logging (Douglas et al. 1993; Douglas et al. 1992). So far, it is not well-understood how habitat loss or alternations will affects riparian anurans on Borneo, but considering the dramatic decline of this group of vertebrates it is expected that biodiversity will decline considerably if ecosystems continue to degrade.

For some species *ex situ* programs may be the only option to avoid extinction (e.g., the Kihansi spray toad, *Nectophrynoides asperginis* [Krajick 2006] or the Panamanian golden frog, *Atelopus zeteki* [Zippel 2002]). Species that are not considered Critically Endangered should be preserved in the wild through protection of key habitats and monitoring. Nevertheless, to identify habitats necessary for survival of a species and subsequent immediate protection requires extensive research and conservations efforts. Captive breeding programs however should be extremely cautious to avoid disease transmission, hence in our project only individuals from the biosecure container facility will be considered for transport to other institutions. *Ex situ* conservation and research programs not only can prevent extinction through captive management and re-introduction to the wild, but offer opportunities for research to identify and, thus, protect key habitats (Zippel et al. 2011).

Conclusion

The species of the genus *Staurois* live and breed along fast-flowing streams and waterfalls. For the first time it was possible to *ex situ* breed two foot-flagging species in captivity and demonstrate the importance of fresh water streams and adjacent gravel pools for reproduction. We suggest that to successfully breed stream dwelling anurans with territorial males/females (also immature juveniles as mentioned previously) performing spacing behaviors (e.g., foot flagging), large and diversely structured terraria, including a waterfall and several options for egg deposition should promise the best success rate for future breeding programs. Further, we emphasize, that zoo-based conservations and research programs help to identify ecological factors that are necessary for the survival of threatened species, and also raise awareness to the ongoing amphibian decline. Public awareness of the conservation needs of threatened amphibian species through zoo-based conservation breeding programs may then be translated into in-range conservation initiatives by regional governments and local stakeholders who are also concerned with the *ex situ* conservation of these two species.

Acknowledgments.—Export and import permission were obtained from the Brunei Museums Department (Reference: 14/JMB/209/68/2) and the Austrian Federal Ministry of Health, respectively. We thank U. Grafe for his continuous professional and logistic help. We are grateful for the dedication and support of R. Riegler, E. Karell, and all other zoo-keepers that are involved in this project. We thank M. Boeckle, N. Potensky, and D. Zupanc for providing their photographs. We also thank the reviewers for valuable comments on the manuscript. The study was supported by the Austrian Science Fund FWF-P22069 and the Society of Friends of the Vienna Zoo.

Author Contributions.—DP carried out the study, analyzed pictures and available data and wrote the manuscript. AW participated in the design of the study and coordinated its implementations at the Vienna Zoo. TW designed and build the breeding facility, carried out the

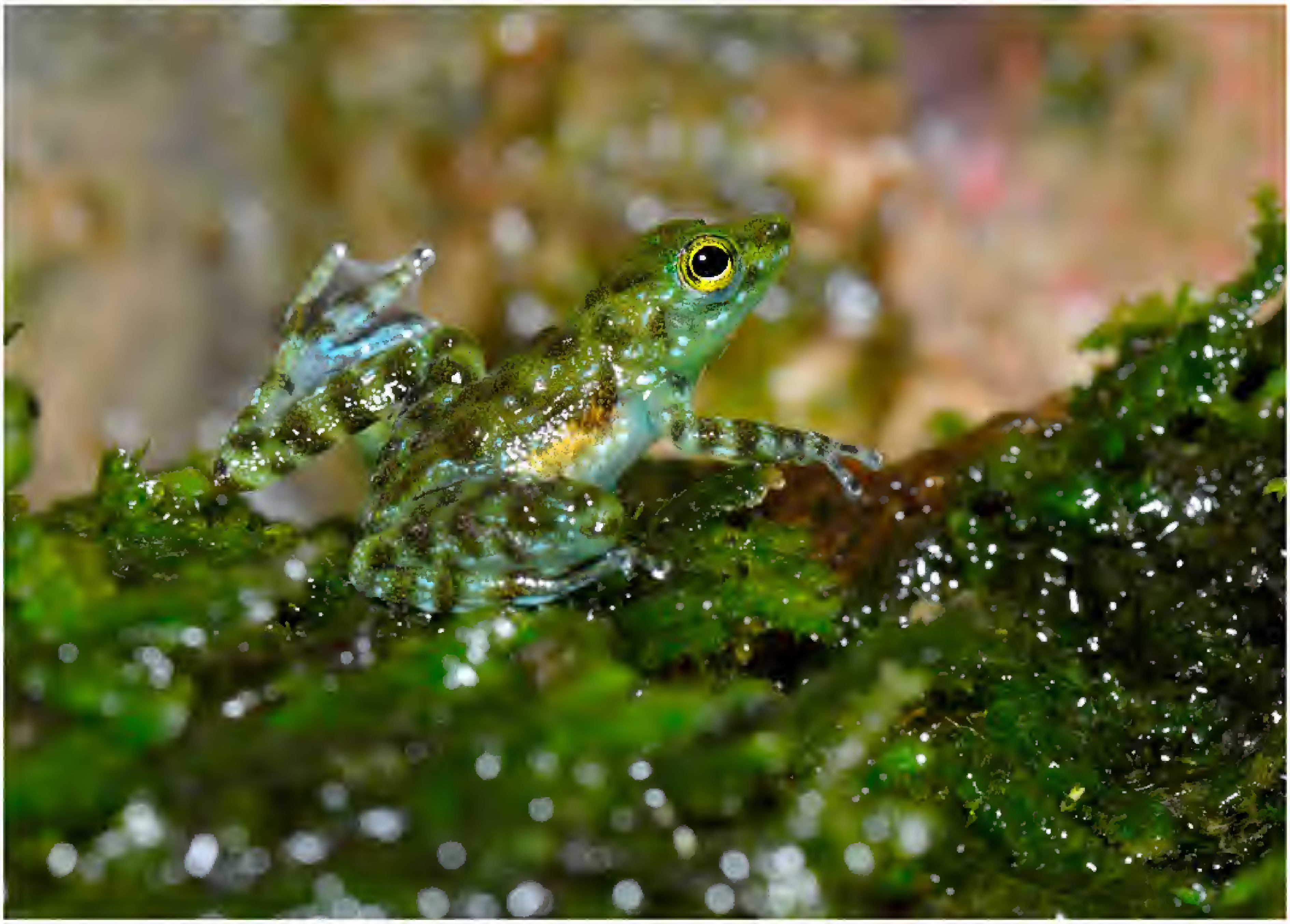


Figure 10. Juvenile *Staurois parvus* performing a foot-flagging behavior. Interdigital webbing are transparent grey and not white as observed in adults (see also Fig. 2). *Image by N. Potensky.*

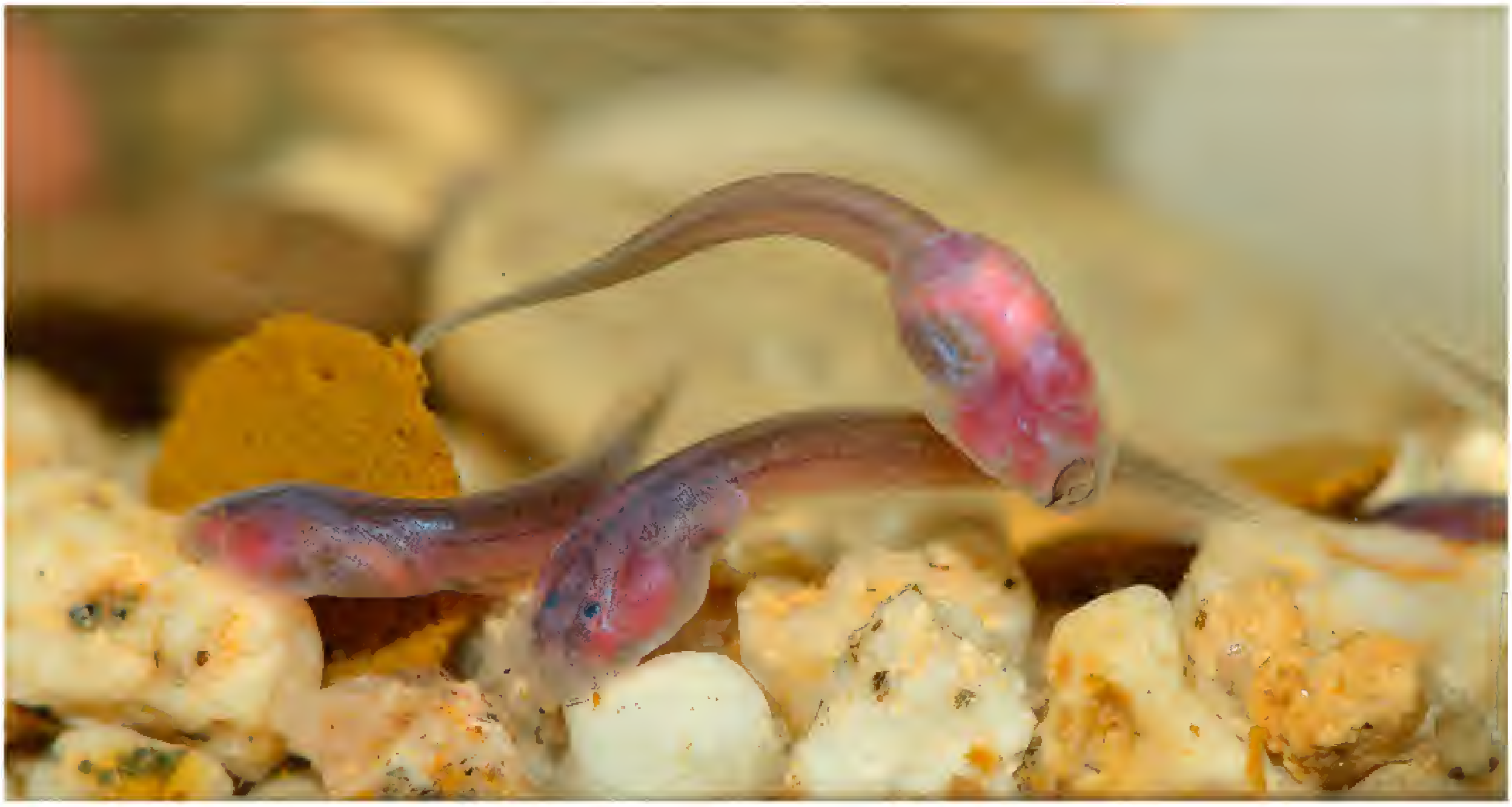


Figure 11. Tadpoles of *Staurois guttatus*. *Image by N. Potensky.*

import of the species, and participated in all decision processes. WH conceived and coordinated the study. All authors read and approved the final manuscript.

Literature cited

Arch VS, Grafe TU, Narins PM. 2008. Ultrasonic signalling by a Bornean frog. *Biological Letters* 4(1):19-22.

Arifin U, Iskandar DT, Bickford DP, Brown RM, Meier R, Kutty SN. 2011. Phylogenetic relationships within the genus *Staurois* (Anura, Ranidae) based on 16S rRNA sequences. *Zootaxa* 2744:39-52.

Boeckle M, Preininger D, Hödl W. 2009. Communication in noisy environments I: Acoustic signals of *Staurois latopalmatus* Boulenger 1887. *Herpetologica* 65(2):154-165.

Browne RK, Wolfram K, Garciá G, Bagaturov MF, Pereboom ZJJM. 2011. Zoo-based amphibian research and conservation breeding programs. *Amphibian and Reptile Conservation* 5(3):1-14.

Douglas I, Greer T, Bidin K, Spilsbury M. 1993. Impacts of rainforest logging on river systems and communities in Malaysia and Kalimantan. *Global Ecology and Biogeography Letters* 3(4/6):245-252.

Douglas I, Spencer T, Greer T, Bidin K, Sinun W, Meng WW. 1992. The impact of selective commercial logging on stream hydrology, chemistry and sediment loads in the Ulu Segama Rain Forest, Sabah, Malaysia. *Philosophical Transactions of the Royal Society of London, Series B: Biological Sciences* 335(1275):397-406.

Gagliardo R, Crump P, Griffith E, Mendelson J, Ross H, Zippel K. 2008. The principles of rapid response for amphibian conservation, using the programmes in Panama as an example. *International Zoo Yearbook* 42(1):125-135.

Gascon C, Collins JP, Moore RD, Church DR, McKay JE, Mendelson JRI. 2007. *Amphibian Conservation Action Plan.* The World Conservation Union (IUCN)/SSC Amphibian Specialist Group, Gland, Switzerland and Cambridge, United Kingdom. 64 p.

Grafe TU, Preininger D, Sztatecsny M, Kasah R, Dehling JM, Proksch S, Hödl W. 2012. Multimodal communication in a noisy environment: A case study of the Bornean rock frog *Staurois parvus*. *PLoS One* 7(5):e37965.

Grafe TU, Wanger TC. 2007. Multimodal signaling in male and female foot-flagging frogs *Staurois guttatus* (Ranidae): An alerting function of calling. *Ethology* 113(8):772-781.

Gururaja KV. 2010. Novel reproductive mode in a torrent frog *Micrixalus saxicola* (Jerdon) from the Western Ghats, India. *Zootaxa* 2642:45-52.

Haas A, Das I. 2012. Frogs of Borneo — The Frogs of East Malaysia and their Larval Forms: An Online Photographic Guide. Zoological Museum Hamburg, Germany. [Online]. Available: http://www.frogsofborneo.org [Accessed: 08 May 2012].

Hödl W, Amézquita A. 2001. Visual signaling in anuran amphibians. In: *Anuran Communication*. Editor, Ryan MJ. Smithsonian Institution Press, Washington, DC, USA. 121-141.

Hoffmann M, Hilton-Taylor C, Angulo A, Böhm M, Brooks TM, Butchart SHM, Carpenter KE, Chanson J, Collen B, Cox NA, Darwall WRT, Dulvy NK, Harrison LR, Katariya V, Pollock CM, Quader S, Richman NI, Rodrigues ASL, Tognelli MF, Vié J-C, Aguiar JM, Allen DJ, Allen GR, Amori G, Ananjeva NB, Andreone F, Andrew P, Ortiz ALA, Baillie JEM, Baldi R et al. 2010. The impact of conservation on the status of the world's vertebrates. *Science* 330(6010):1503-1509.

Inger RF. 1969. Organization of communities of frogs along small rain forest streams in Sarawak. *Journal of Animal Ecology* 38(1):123-148.

Inger R, Iskandar D, Das I, Stuebing R, Lakim M, Yambun P. 2004. IUCN Red List of Threatened Species. [Online]. Available: www.iucnredlist.org [Accessed: 16 April 2012].

Inger RF, Stuebing RB. 2005. *A Field Guide to the Frogs of Borneo.* (Second Edition). Natural History Publications (Borneo) Sdn. Bhd. Kota Kinabalu, Sabah, Malaysia. 201p.

Inger RF, Tan FL. 1996. Checklist of the frogs of Borneo. *Raffles Bulletin of Zoology* 44(2):551-574.

Inger RF, Voris HK. 1993. A comparison of amphibian communities through time and from place to place in Bornean forests. *Journal of Tropical Ecology* 9(04):409-433.

Inger RF, Wassersug RJ. 1990. A Centrolenid-Like Anuran Larva from Southeast Asia. *Zoological Science* 7(3):557-561.

Iskandar DT, Colijn E. 2000. Preliminary checklist of Southeast Asian and New Guinean amphibians. *Treubia* 31:1-134.

IUCN. 2011. IUCN Red List of Threatened Species. [Online]. Available: www.iucnredlist.org [Accessed: 16 April 2012].

Keller A, Rödel M-O, Linsenmair KE, Grafe TU. 2009. The importance of environmental heterogeneity for species diversity and assemblage structure in Bornean stream frogs. *Journal of Animal Ecology* 78(2):305-314.

Konopik O. 2010. Movement patterns, habitat use and diet of tropical ranid frogs: A comparison between pioneer and native anurans in Borneo. M.S. Thesis, University of Wuerzburg, Wuerzburg, Germany, Department of Animal Ecology and Tropical Biology. 96 p.

Krajick K. 2006. The lost world of the Kihansi toad. *Science* 311(5765):1230-1232.

Lee S, Zippel K, Ramos L, Searle J. 2006. Captive-breeding programme for the Kihansi spray toad *Nectophrynoides asperginis* at the Wildlife Conservation

Society, Bronx, New York. *International Zoo Yearbook* 40(1):241-253.

Malkmus R, Kosuch J, Kreutz J. 1999. Die larve von *Staurois tuberilinguis* Boulenger, 1918. Eine neuecentroleniden Kaulquappe aus Borneo (Anura: Ranidae). *Herpetozoa* 12(1/2):17-22.

Matsui M, Mohamed M, Shimada T, Sudin A. 2007. Resurrection of *Staurois parvus* from *S. tuberilinguis* from Borneo (Amphibia, Ranidae). *Zoological Science* 24(1):101-106.

McFadden M, Duffy S, Harlow P, Hobcroft D, Webb C, G W-F. 2008. A review of the green and golden bell frog *Litoria aurea* breeding program at Taronga Zoo. *Australia Zoologist* 34(3):291-296.

Narvaes P, Rodrigues MT. 2005. Visual communication, reproductive behavior, and home range of *Hylodes dactylocinus* (Anura, Leptodactylidae). *Phyllomedusa* 4(2):147-158.

Preininger D, Boeckle M, Hödl W. 2007. Comparison of anuran acoustic communities of two habitat types in the Danum Valley Conservation Area, Sabah, Malaysia. *Salamandra* 43(3):129-138.

Preininger D, Boeckle M, Hödl W. 2009. Communication in noisy environments II: Visual signaling behavior of male foot-flagging frogs *Staurois latopalmatus*. *Herpetologica* 65(2):166-173.

Stuart SN, Chanson JS, Cox NA, Young BE, Rodrigues ASL, Fischman DL, Waller RW. 2004. Status and trends of amphibian declines and extinctions worldwide. *Science* 306(5702):1783-1786.

Zimmerman B, Simberloff D. 1996. An historical interpretation of habitat use by frogs in a Central Amazonian Forest. *Journal of Biogeography* 23(1):27-46.

Zippel K. 2002. Conserving the Panamanian golden frog: Proyecto Rana Dorada. *Herpetological Review* 33(1):11-12.

Zippel K, Johnson K, Gagliardo R, Gibson R, McFadden M, Browne R, Martinez C, Townsend E. 2011. The Amphibian Ark: A global community for *ex situ* conservation of amphibians. *Herpetological Conservation and Biology* 6(3):340-352.

Received: 12 May 2012
Accepted: 26 June 2012
Published: 7 September 2012

Doris Preininger has already worked with foot-flagging frogs in her undergraduate studies. In her graduation thesis she addresses the multimodal (acoustic and visual) communication in anurans and tries to explain how selection on senders and receivers promotes complex displays under different acoustic and environmental conditions. She is currently completing her dissertation at the Department of Evolutionary Biology, University Vienna. Her research includes foot-flagging species from Borneo and India and focuses on a bio-acoustic and experimental approach in the natural habitat of the respective species. In several visits to Borneo it became quite obvious to her that agricultural demands gradually degrade the primary forest and that every conservation effort possible should be immediately taken to conserve and protect the biodiversity of the rainforest.

Anton Weissenbacher is Zoological Curator at Vienna Zoo, committee member of the European Association of Aquariums and coordinator of the European StudBook (ESB) of *Brachylophus fasciatus*. At Vienna Zoo he is responsible for the zoological and technical management of the aquarium, the "Desert house," the "Rainforest house," and monitors all zoo issues concerning fishes, amphibians, reptiles, and invertebrates. Under his zoological guidance, the zoo has recently registered several exceptional breeding successes such as the world's first Northern river terrapin, *Batagur baska*, hatched in captivity. Together with his team he manages the world's largest *Aphanius* species breeding group. He has supervised various scientific publications and has initiated several conservation projects including Project *Batagur baska*.

Thomas Wampula has worked since 1996 at the Vienna Zoo Schönbrunn. He started as Animal Care Taker at the Aquarium-house and later transferred to the "Rainforest house" where his first and foremost interests were amphibians, reptiles, and fish. His duties and responsibilities included the arrangement and design of terraria and the maintenance of facilities. In 2007 he became a member of the Department of Technology and Project Development at the zoo and now is engaged in planning, design, and development of viviaria in the entire Vienna Zoo. The foot-flagging frog project has repeatedly led him to Borneo, where he assisted in field work, capture, transport, and care of frogs, and at the zoo he managed the construction of the breeding facility.

Walter Hödl has an international record in a wide range of topics in amphibian ecology and behavior. Since 1997 he has worked as an Associate Professor at the Institute of Zoology, University of Vienna. During the last years, he has studied numerous foot-flagging frog species in Asia, Australia, and South America and has established the South-East Asian frog genus *Staurois* spp. as a research model. Prework on visual signaling frog species began more than 10 years ago, when he documented for the first time in a scientific film[1]—together with Brazilian colleagues—anuran foot-flagging behavior, and later compared visual signal repertoires of anuran species worldwide. He discovered the use of the vocal sac as a visual signal independently of sound production in *Phrynobatrachus krefftі*, and set off a study on color change in the explosively breeding anuran species *Rana arvalis*. In the neotropics, his so called "handy fellow" *Allobates femoralis* has been his research focus over the past 30 years and has led to numerous research and teaching visits to Brazil (Universities at Belém, São Luís João Pessoa, Manaus, São Paulo, Rio Branco, Ribeirão Preto, Feira da Santana, and at MPEG Belem, INPA Manaus) and Peru and French Guiana, enabling him to spend over eight years of fieldwork in Amazonia. Among many functions, he is a member of the scientific committee of WWF Austria and the head of the nature conservation society of lower Austria and continuously establishes cooperation around the globe to promote anuran research and conservation.

---

[1] Hödl W, Rodrigues MT, Accacio de M, Lara PH, Pavan D, Schiesari LC, Skuk G.1997. Foot-flagging display in the Brazilian stream-breeding frog *Hylodes asper* (Leptodactylidae). Austrian Federal Institute of Scientific Film (Film CTf 2703 ÖWF Wien). [web application]. 2012. AmphibiaWeb, Berkeley, California. [Online]. Available: http://amphibiaweb.org/cgi/amphib_query?where-genus=Hylodes&where-species=asper [Accessed: 02 July 2012].

Copyright: © 2012 Edmonds et al.This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs 3.0 Unported License, which permits unrestricted use for non-commercial and education purposes only provided the original author and source are credited.

Amphibian & Reptile Conservation 5(3):57-69.

Building capacity to implement conservation breeding programs for frogs in Madagascar: Results from year one of Mitsinjo's amphibian husbandry research and captive breeding facility

[1,7]Devin Edmonds, [1,8]Justin Claude Rakotoarisoa, [1]Rainer Dolch, [2]Jennifer Pramuk, [2,3]Ron Gagliardo, [4,5]Franco Andreone, [4]Nirhy Rabibisoa, [4]Falitiana Rabemananjara, [6]Sahondra Rabesihanaka, and [6]Eric Robsomanitrandrasana

[1,7]Association Mitsinjo, Andasibe, MADAGASCAR [2]Woodland Park Zoo, Seattle, Washington, USA [3]Amphibian Ark, Apple Valley, Minnesota, USA [4]Amphibian Specialist Group, MADAGASCAR [5]Museo Regionale di Scienze Naturali, Turin, ITALY [6]La Direction Générale des Forêts, MADAGASCAR

***Abstract.*—Madagascar is ranked 12th in amphibian species richness by the International Union on the Conservation of Nature (IUCN) and is considered to be one of the highest priority countries for amphibian conservation. Nearly one quarter of the island's amphibian species are threatened with extinction with habitat alteration and over-harvesting for the pet trade contributing most to this dramatic decline. The impending threat of the amphibian chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*), which has been associated with many of the world's recent amphibian population declines and extinctions, is of great concern. In response to the tremendous threats facing Madagascar's amphibians, a national strategy for amphibian conservation was developed, emphasizing the need for *ex situ* conservation action. This project was officially launched through a collaborative effort between a community-run organization, the IUCN, and the Malagasy government. With significant financial support from multiple international agencies, the result was the construction of a captive breeding facility in Andasibe, east-central Madagascar. We discuss the process for developing and implementing this project which has included facility construction, terrarium building, culturing local feeder insects, and the training of Malagasy technicians. This is the first captive breeding and amphibian conservation project of its kind in Madagascar. Our hope is that it will not only serve as a model for other range country facilities, but become a center for training and education in an area of Madagascar that contains tremendous amphibian diversity and endemism.**

Key words. Amphibians, Madagascar, husbandry, capacity building, frogs, breeding facility, live food colonies

Citation: Edmonds D, Rakotoarisoa JC, Dolch R, Pramuk J, Gagliardo R, Andreone F, Rabibisoa N, Rabemananjara F, Rabesihanaka S, Robsomanitrandrasana E. 2012. Building capacity to implement conservation breeding programs for frogs in Madagascar: Results from year one of Mitsinjo's amphibian husbandry research and captive breeding facility. *Amphibian & Reptile Conservation* 5(3):57-69(e55).

Introduction

With more than 286 described frog species (AmphibiaWeb 2012), Madagascar supports among the highest amphibian species richness of any country in the world. All but one frog species are endemic, while salamanders, and caecilians are unknown from the island. The diversity of frog species is highest in the eastern rainforest belt (Andreone et al. 2005), with the area around the village of Andasibe in east-central Madagascar being particularly speciose, supporting more than 100 frog species within a 30 km radius of town (Dolch 2003).

The amphibian faunae around Andasibe and elsewhere in Madagascar is especially amazing in terms of their ecological, morphological, and reproductive diversity (Andreone et al. 2008). For example, the more than 120 species in the subfamily Mantellinae interestingly do not engage in amplexus, and a number exhibit varying forms of parental care. Members of the genus *Mantella* are toxic and display bright aposematic coloration serving as a familiar example of convergent evolution with the poison frog family Dendrobatidae from Central and South America. Containing some of the smallest frogs in the world, species in the genus *Stumpffia* deposit small numbers of eggs in terrestrial foam nests where non-feeding tadpoles develop directly into frogs. The biodiversity of Madagascar is truly impressive, not only in terms of its well-known lemur and plant species, but also in the behavioral and morphological attributes of its diverse amphibian fauna.

Correspondence. *Email: [7]devin@amphibiancare.com, [8]babakotokely@gmail.com*

Unfortunately, nearly one quarter of Madagascar's amphibian species are considered threatened with extinction, and an additional 18.5% of species have not yet had their conservation status determined and are listed as Data Deficient (IUCN 2011). The most significant threat facing the frogs of Madagascar is habitat alteration (Andreone et al. 2005; Glaw and Vences 2007), largely due to agricultural activities, charcoal production, logging, and both artisanal and large-scale industrial mining operations. Additionally, particularly charismatic and colorful frog species, such as those in the genera *Dyscophus*, *Mantella*, and *Scaphiophryne*, are at risk from over-harvesting for the international pet trade (Andreone et al. 2006). Of special concern are the Malagasy frog species confined to high altitudes due to the pressing threat of global warming and upslope elevational displacement (Raxworthy 2008).

The threat of emergent infectious diseases is also of grave concern. The amphibian chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*), which has been associated with drastic population declines and extinctions elsewhere in the world, until recently was thought to be absent from Madagascar (Weldon et al. 2008). However, recent indications of *Bd* in the Makay region still remain unconfirmed (Rabemananjara et al. 2011; Andreone et al. 2012). Lötters et al. (2011) conducted an extinction risk assessment based on a combination of environmental models and an examination of species life history traits, and revealed that many of the frog species in Madagascar are likely to be severely affected by *Bd*. Considering this, it is vital to take appropriate biosecurity precautions, develop awareness campaigns, and enact necessary conservation measures as quickly as possible before *Bd* spreads throughout the country.

Captive breeding can be used as a tool for the conservation of amphibians by establishing captive assurance colonies when threats cannot be addressed in time to prevent extinction, and by developing associated reintroduction and population supplementation programs for species in decline (Griffiths and Pavajeau 2008; Mendelson et al. 2007). In recent years, *ex situ* conservation measures for amphibians have notably been applied in direct response to the threat of *Bd* (Pessier 2008). The Amphibian Ark was formed in 2006 to build capacity in range country and subsequently has assembled many tools for helping implement *ex situ* programs (Zippel et al. 2011). Though these programs have limitations and are temporary solutions, in some cases they are the only option available to prevent imminent extinction (Pavajeau et al. 2008).

There are many urgent threats to the endemic frog species in Madagascar, but as of yet there is little capacity to address them through *ex situ* means. A recent survey by García et al. (2008) of zoological institutions and private breeders around the world found only 27 species of frogs from Madagascar were being kept in captivity, and of these barely more than half (14 species) had reproduced in the last ten years. Furthermore, these programs are largely informal, operating without proper bio-security and population management practices, which are crucial to the long-term success of projects supplying animals for future reintroduction efforts. This knowledge gap and lack of capacity hinders *ex situ* conservation measures. Additionally, until recently, expertise in amphibian husbandry remained outside of Madagascar and this prohibited the development of in-country captive breeding programs. Developing captive breeding programs within the native range of a species is advantageous for numerous reasons, including significantly reducing biosecurity risks, lowering financial costs when compared to exporting species for breeding programs elsewhere, and instilling pride and confidence in range country stakeholders (Gagliardo et al. 2008).

Methods and implementation

ACSAM

To develop a plan to address the threats facing the amphibians of Madagascar, a conference of more than 100 international and Malagasy experts was held in Antananarivo in September, 2006. Known as "A Conservation Strategy for the Amphibians of Madagascar" (ACSAM), this conference led to the development of the *Sahonagasy Action Plan* (Andreone and Randriamahazo 2008) which is now the national strategy for amphibian conservation in Madagascar, endorsed and supported by the Malagasy government. Within this action plan was a call urging a proactive approach to be taken to develop husbandry expertise for frog species from varied ecological guilds, which had yet to be kept in captivity. This would facilitate rapid *ex situ* conservation action should the need arise.

Following ACSAM, the community-run conservation organization Mitsinjo developed a plan to establish a biosecure facility specifically for the purpose of building capacity to maintain, breed, and conserve local amphibian species. Based in the frog diversity hotspot of Andasibe, Mitsinjo is involved in a varied set of activities including research, rainforest restoration, environmental education, ecotourism, and community health components. The organization is composed of approximately 40 members from the Andasibe population, about a dozen of which are employed fulltime.

Mitsinjo identified three main objectives for the breeding facility:

1) Build capacity within Mitsinjo and train technicians to care for and manage captive frog populations. Share knowledge and expertise gained with other organizations and institutions in Madagascar.

2) Conduct husbandry research on local frog species from varied ecological guilds to understand their life

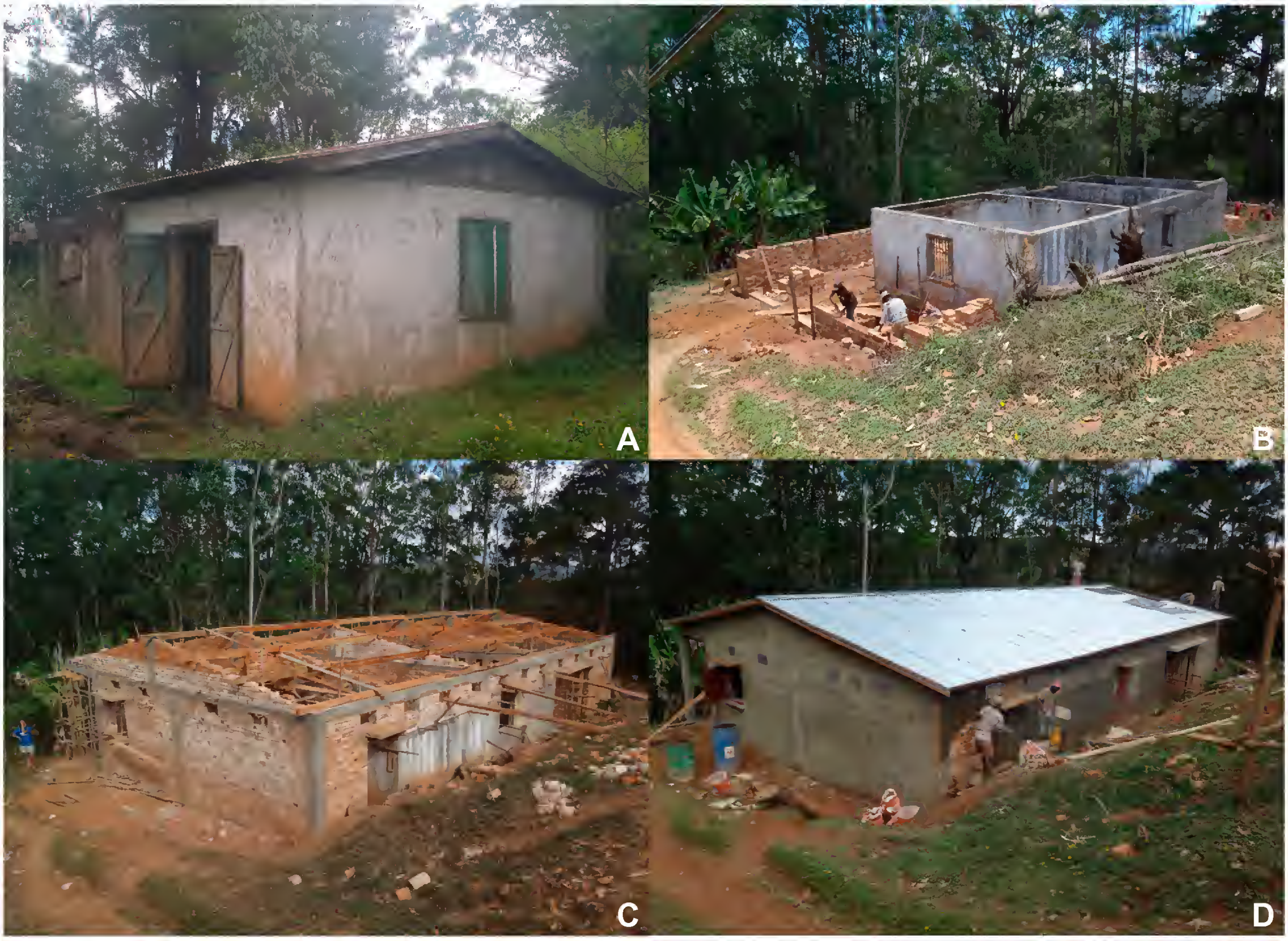


Figure 1. The facility was constructed between November 2010 and March 2011 from the foundations of an old abandoned forest station. A) Original abandoned building in January 2009. B) Facility construction November 2010. C) Facility construction December 2010. D) Facility construction January 2011.

histories and captive care requirements, facilitating *ex situ* conservation efforts.

3) Establish captive assurance colonies of threatened frog species from the Andasibe-area and develop associated reintroduction and supplementation programs lest they are needed.

Facility specifications and construction

Fundraising began in 2009 and was received first from Amphibian Ark, the Wildlife Conservation Society, and the Association of Zoos and Aquariums. Facility construction began in November 2010, with the basic infrastructure of the building being completed in March 2011 (Figure 1). The facility was constructed in the Mitsinjo-managed Analamazaotra Forest Station from the foundations of an abandoned building historically used for forestry activities. The location was chosen for its elevated position to prevent flooding during the cyclone season and for the ease of access to the main road leading to Andasibe village.

Measuring 185 m^2, the facility contains three separate areas for live food production, captive breeding and husbandry research, and an isolated room for quarantine (Figure 2). Entrance to the facility is through two sets of doors, in between which is a threshold on the floor to help prevent organic debris from entering. Beyond the barrier is a hand washing station and area to change into dedicated clothing and footwear. The building was designed to facilitate workflow habits that minimize biosecurity risks, with staff from Amphibian Ark, Woodland Park Zoo, North-West University, and Jersey Zoo contributing input during construction based on experience gained designing similar facilities elsewhere in the world.

Frog species kept at the facility are and will be composed of a local species assemblage, considerably lowering biosecurity risks (Pessier and Mendelson 2010). Water is sourced from a river at Ambatomandondona, which is 2.5 km from the facility. This source is supplemented with rainwater. A solar water heater, 1μ sediment filter, and carbon filtration will be used to help prevent amphibian pathogens from entering the facility through the water supply. Additionally, all windows, doors, and drains are sealed to prevent pests and amphibians from entering or exiting the building. Wastewater is discharged through a carbon and sediment filter to stop soaps, detergents, and chemicals used for cleaning and disinfecting materials from polluting the surrounding forest.

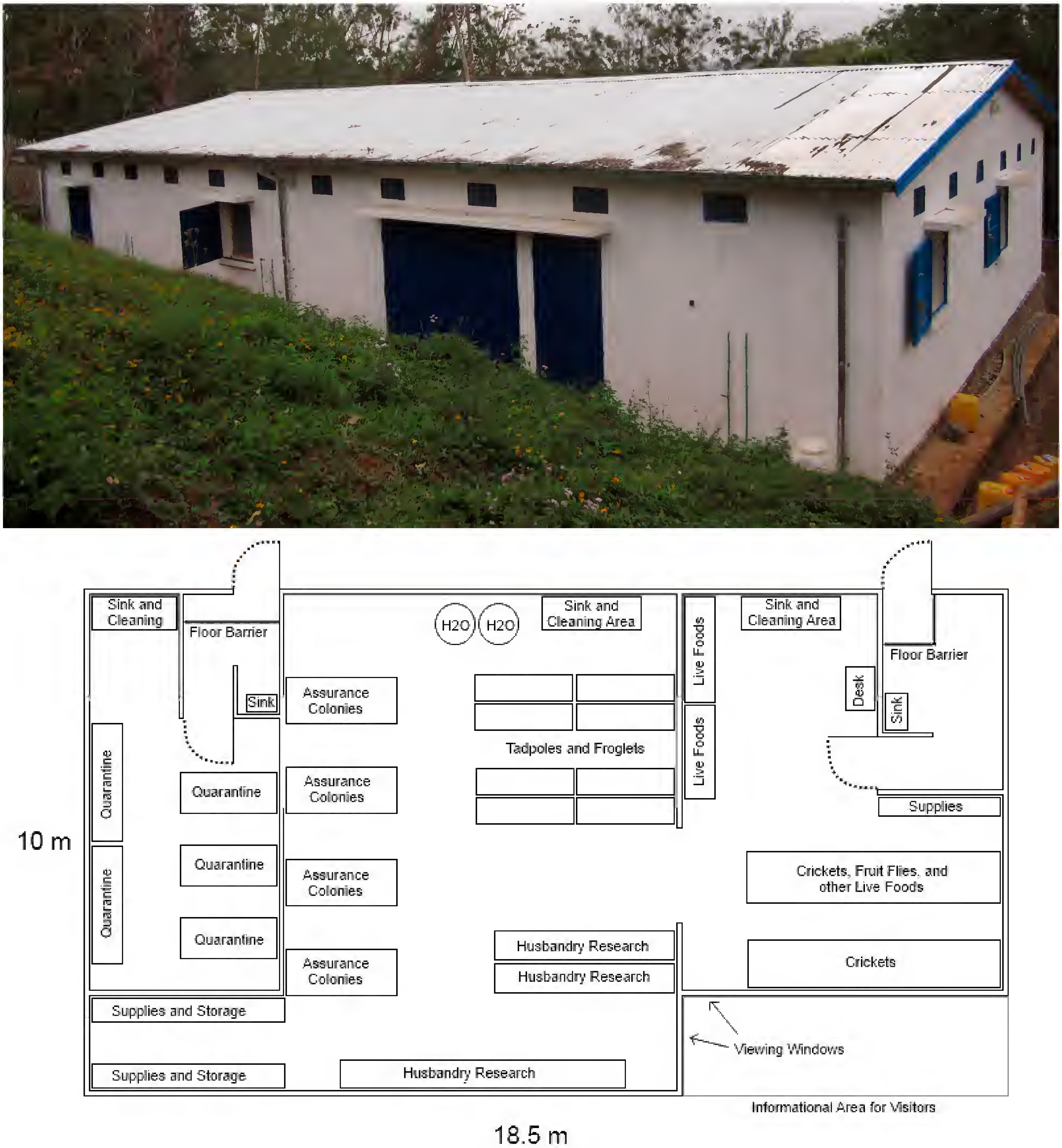

Sink and Cleaning
Floor Barrier
Sink
Quarantine
Quarantine
Quarantine
Quarantine
Quarantine
Assurance Colonies
Assurance Colonies
Assurance Colonies
Assurance Colonies
H2O
H2O
Sink and Cleaning Area
Tadpoles and Froglets
Husbandry Research
Husbandry Research
Husbandry Research
Supplies and Storage
Supplies and Storage
Live Foods
Live Foods
Sink and Cleaning Area
Desk
Sink
Floor Barrier
Supplies
Crickets, Fruit Flies, and other Live Foods
Crickets
Viewing Windows
Informational Area for Visitors
10 m
18.5 m

Figure 2. Overview of the biosecure Mitsinjo amphibian captive breeding and husbandry research center as of April 2012.

Materials to build shelves and terraria (wood, glass, silicone, aluminum, screen, etc.) were all sourced from within Madagascar, and were constructed locally in Andasibe. Material used inside terraria such as gravel, dead leaves, and live plants were collected from the surrounding forest when possible. Plants were disinfected with a 0.5% sodium hypochlorite solution before entering the facility, with other organic material being cleaned with water and then fully air dried in the sun for several days prior to being brought inside.

Twenty-four terraria are currently used for rearing tadpoles and offspring with an additional 46 terraria constructed and being used for adult frogs (Figure 3). Terraria are setup in an "open-system" where they are outfitted with bulkheads that drain into floor drains. This allows terraria to be cleaned and serviced without needing to be moved off of shelving units, and helps regulate the moisture content of the substrate. Wastewater from terraria housing captive assurance populations and from terraria for husbandry research drain into separate floor

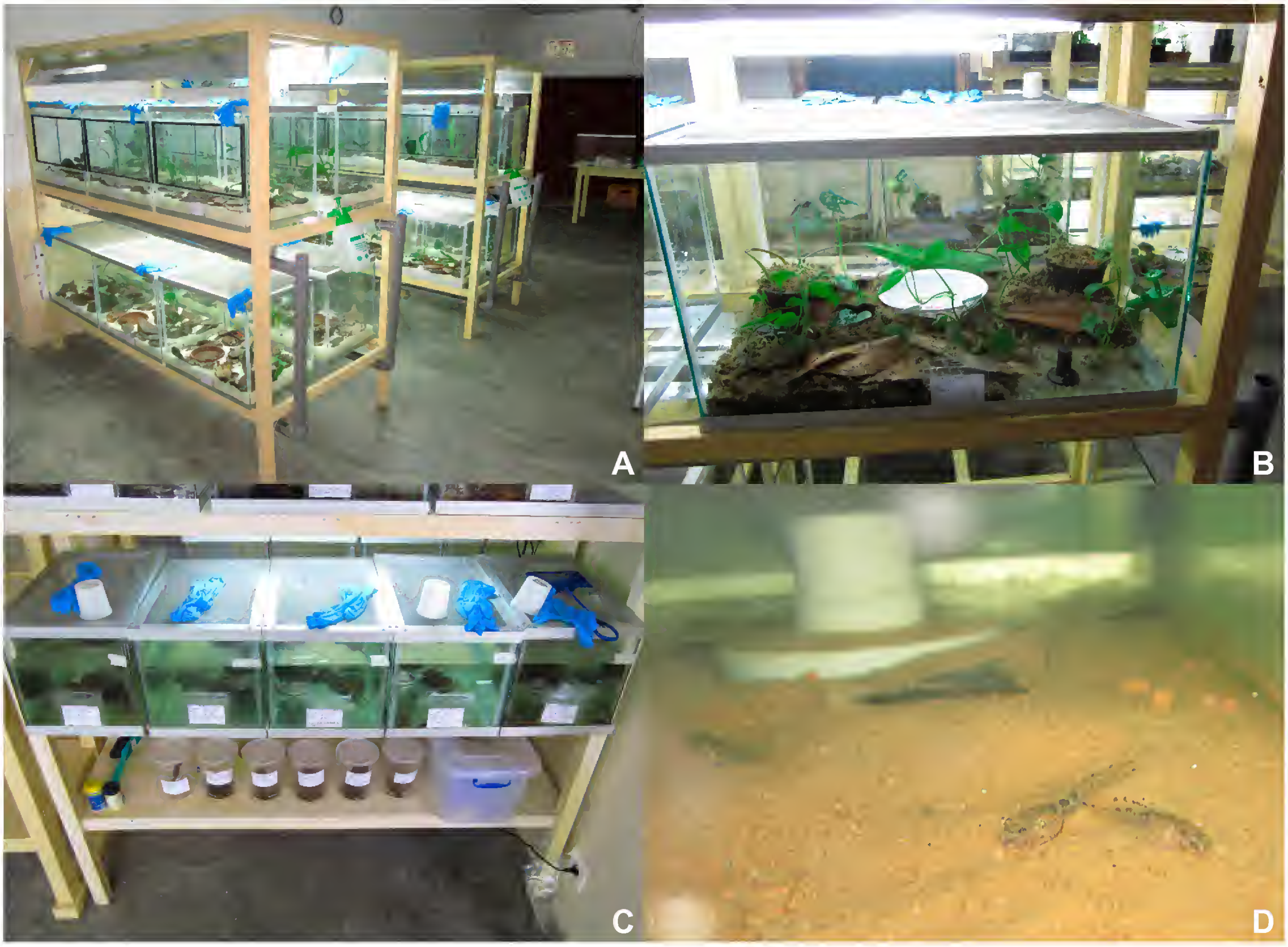


Figure 3. Terraria and aquaria at the breeding facility. A) Terraria setup on shelving and plumbed so wastewater flows into a drain in the floor. B) A terrarium housing a group of *Boophis pyrrhus*. C) Aquaria for raising tadpoles. D) *Boophis pyrrhus* tadpoles produced at the facility.

drains. The facility has capacity and is planned to support a total of 300+ terraria and aquaria, which are continually being built by Mitsinjo and should be finished in 2013.

Mitsinjo's project was officially launched through a Contract of Collaboration with the IUCN SSC Amphibian Specialist Group (ASG) of Madagascar and the Malagasy governmental agency Direction Générale des Forêts (DGF) in April 2011. This contract ensures all activities comply with Malagasy Law and helps make certain Mitsinjo's objectives complement and correspond to those in the *Sahonagasy Action Plan*.

Frog and live food sources

All live foods produced at the facility were originally collected from around Andasibe to prevent introducing potentially invasive invertebrate species to the area. Live food species identification was provided by the University of Antananarivo Department Of Entomology. While the facility was being constructed, more than six months were spent collecting local invertebrates and developing techniques to culture them in captivity. Mitsinjo continues to expand live food sources to provide variation in the diet of the captive frog populations. Early on, advisors to the project stressed the importance of establishing live food colonies before frogs were brought into captivity. Four frog species were collected and acclimated to captivity in April 2011 once live food cultures were established and the Contract of Collaboration between Mitsinjo, ASG, and the DGF was finalized. The first frogs were assigned to six groups in separate terraria (Table 1). Species were chosen not only for their husbandry research potential, but also to provide Mitsinjo technicians with varied practical experiences caring for taxa with diverse

Table 1. Initial breeding groups established for training in April, 2011.

Group	Species	Males to Females	Breeding?
BLBL-A	*Blommersia blommersae*	5.0	No
BLBL-B	*Blommersia bommersae*	5.0	No
BOPY-A	*Boophis pyrrhus*	3.1	Yes
HEBE-A	*Heterixalus betsileo*	2.1	No
MABE-A	*Mantidactylus betsileanus*	3.2	Yes
MABE-B	*Mantidactylus betsileanus*	4.2	Yes

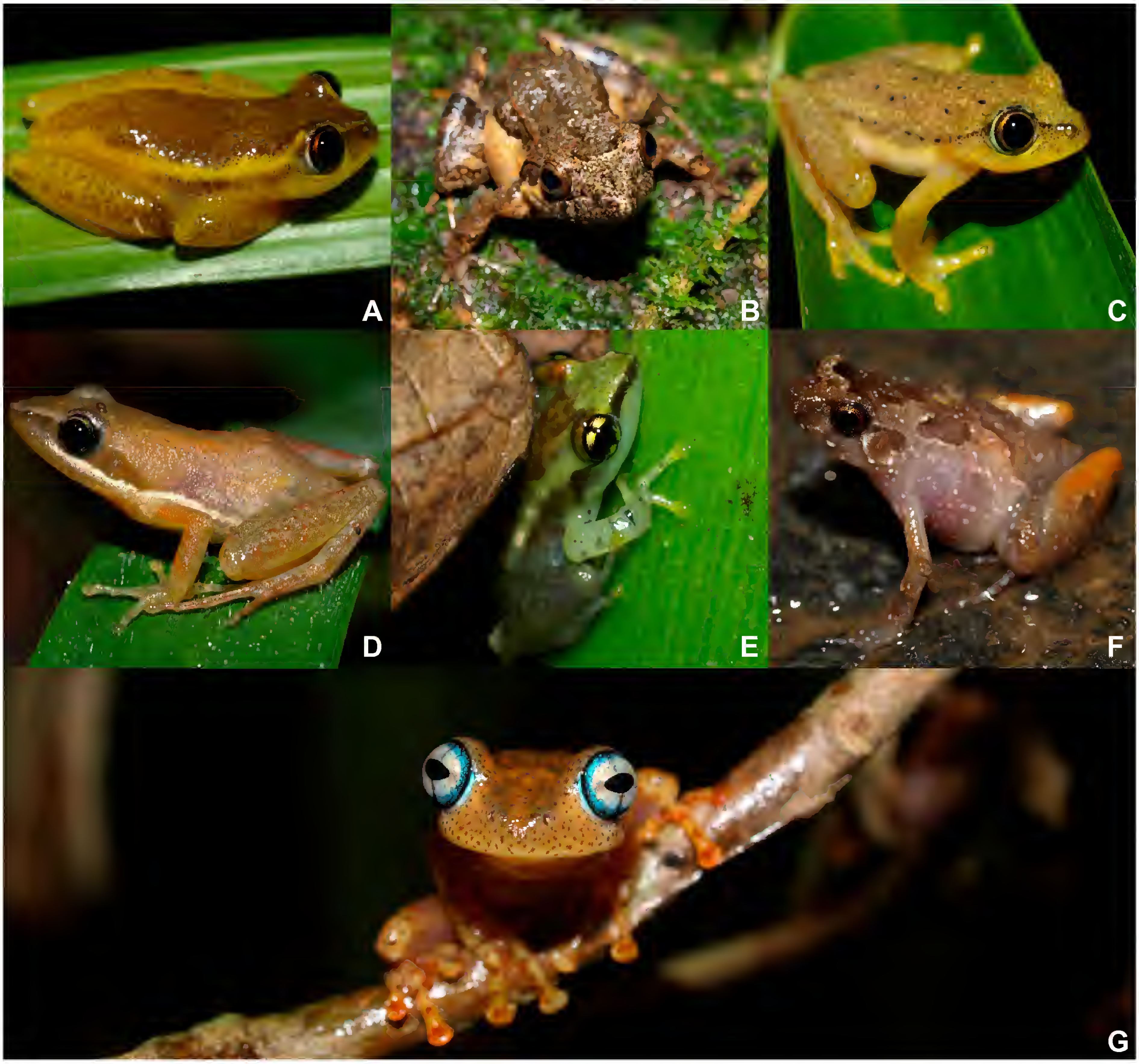


Figure 4. Seven species of frogs were included in a husbandry research and technician training program during the first year of the project. The IUCN Red List status, in parenthesis, follows species. A) *Heterixalus betsileo* (LC). B) *Mantidactylus betsileanus*, (LC). C) *Heterixalus punctatus* (LC). D) *Blommersia blommersae* (LC). E) *Guibemantis* aff. *albolineatus* "Andasibe" (DD). F) *Stumpffia* sp. "Ranomafana" (DD). G) *Boophis pyrrhus* (LC).

life histories and, presumably, different captive care requirements. Additional individuals of the first four species as well as three new species were enrolled in the program throughout the following year, totaling seven species being kept for training and research as of June, 2012 (Figure 4).

All frogs were collected from or near the road leading to Andasibe village. Two days were spent searching for and collecting target species, after which all frogs were moved into the quarantine room for housing while the final aspects of construction in the main frog room were completed. Body score condition of each individual was recorded weekly during acclimation.

The second group of frogs acclimated to captivity in 2012 was weighed upon entry into and exit out of quarantine. Only after all appeared in good condition, and there were no unexplained mortalities, were the frogs from the second group moved to the same room, where established populations were being maintained. Detailed records to track their individual identities and sex, health in captivity, collection location, and breeding history are managed in a studbook by Mitsinjo, ASG-Madagascar, and the DGF.

Species currently kept for husbandry research at the facility have either an IUCN Red List status of Least Concern (LC) or Data Deficient (DD), and are not considered priority species for rescue operations by Amphibian Ark. The decision to work with locally abundant LC or DD species was made to manage risks while technicians gained the specialized knowledge and practical experience needed to maintain captive frog populations in a biosecure conservation breeding facility. Information

Figure 5. Lectures and discussions during January-March 2011 helped train Mitsinjo technicians in captive frog husbandry techniques.

Figure 6. A practical hands-on lesson in terraria design and construction, early March, 2011.

and experience gained from maintaining these non-priority species may be applied to establishing captive assurance colonies and developing population supplementation or reintroduction programs for highly threatened species in the future.

Results and discussion

Mitsinjo technician training

To assemble a team of Mitsinjo technicians dedicated to the daily husbandry of amphibians and live food colonies at the facility, a week-long training course was developed in January 2011, which included presentations about basic amphibian biology, ecology, and captive husbandry techniques.

From a group of 14 Mitsinjo members who participated in this initial training course, five technicians were selected to work at the facility and were enrolled in a further two months of intensive preparation with the project's director. Training was composed of assigned readings and related activities about amphibian husbandry, as well as practical lessons involving caring for newly established live food colonies, building terraria, and identifying and handling frog species in the field. As a final component of the training program, a week of on-site presentations and demonstrations about frog husbandry was presented by staff from the Woodland Park Zoo and Amphibian Ark (Figures 5 and 6).

One of the objectives of the project is to build capacity within other Malagasy institutions and organizations to help develop additional amphibian conservation breeding programs elsewhere in Madagascar. As a first step in this direction, a live food production training course supported by Durrell Wildlife Conservation Trust was carried out by Mitsinjo in November 2011 for the University of Antananarivo's Department of Animal Biology. During this week-long course, Mitsinjo technicians instructed a group from the university in techniques developed to culture local invertebrate species. The newly trained university technicians returned to Antananarivo with starter cultures of live foods to practice culturing them in their laboratory, thereby developing the first set of skills needed to maintain captive frog populations.

Live food production

Fruit flies

Fruit flies (*Drosophila* spp.) were the first live foods established by Mitsinjo, with the earliest successful cultures produced in October, 2010. Two species of different sizes were initially captured, however, only the smaller species (similar in size to the familiar *Drosophila melanogaster*) proved easily cultured. Plastic water bottles covered with fabric secured in place with rubber bands are used to contain the flies (Figure 7), with media being composed entirely of ingredients available locally in Andasibe (Table 2).

Table 2. Fruit fly media (makes 10 cultures)

Ingredient	Quantity
Potatoes–boiled until soft	12-15
Bananas	2
Powdered milk	6 tablespoons
Sugar	2 tablespoons
Baker's yeast	~20-40 granules per culture

Crickets

Trial cricket breeding began in November 2010. Five different species including *Gryllodes sigillatus*, one *Gryllus* sp., two *Modicogryllus* sp., and a cave cricket of the family Rhaphidophoridae have been bred by Mitsinjo (Figure 8), but only three are currently producing in quantities large enough to feed captive frogs. Crickets are maintained in ventilated plastic boxes labeled with the hatch date and the species. Boxes measure 60L × 40W ×

30H cm for adult breeders and 35L × 25W × 20H cm for juveniles. The boxes are stored on shelves heated with heat cable which is attached to a thermostat. The temperature varies with season, but typically is maintained between 22 °C and 27 °C. Breeding slowed considerably in 2011 during the cool months of July and August, during which time the facility did not yet have electricity for heating, and nighttime temperatures dropped to as low as 13 °C. Crickets are fed a varied diet of seasonally-available fruits and vegetables (carrot, zucchini, apple, potato, mango, cucumber, etc.) as well as a protein source of ground *patsamena* (a small dried shrimp widely available at markets in Andasibe).

Springtails

The first springtails (*Collembola* sp.) cultured at the facility were sourced from bark on a mango tree in Andasibe village in April, 2011. Attempts were made to culture them on multiple substrates including dead leaves, a soil mixture, and charcoal. Moist charcoal proved to be the most practical. To determine the best food source for the springtails, cultures were divided into two different groups, one fed ground *patsamena* and the other fed Aquafin Professional Basic Fish Flake. Cultures fed fish flake were substantially more productive.

Other live food sources

In addition to fruit flies, crickets, and springtails, Mitsinjo has attempted to establish cultures of various other invertebrates from the Andasibe-area. The most success has been with a local cockroach species from the forest which cannot fly or climb smooth surfaces. They are cared for in nearly an identical way to crickets but are fed a slightly different diet which includes powdered milk. Up to now, only four individuals have been found and collected, and from these founders breeding has only occurred twice, first in October 2011 and then again in January 2012. Currently, Mitsinjo is maintaining a colony of around 60 roaches, most of which are still juveniles. It is expected to take at least one additional year before they are producing enough to be used as a food source for captive frogs.

There has been some success in culturing isopods. These were setup in small plastic boxes layered with moist cardboard and leaf litter, and were fed fish flake. The isopods survived and even appeared to reproduce, but for unknown reasons, all cultures died between June and September 2011. In the future, Mitsinjo plans to again collect isopods and start new cultures.

A small beetle species was also cultured for food. These were originally sourced in grains purchased at

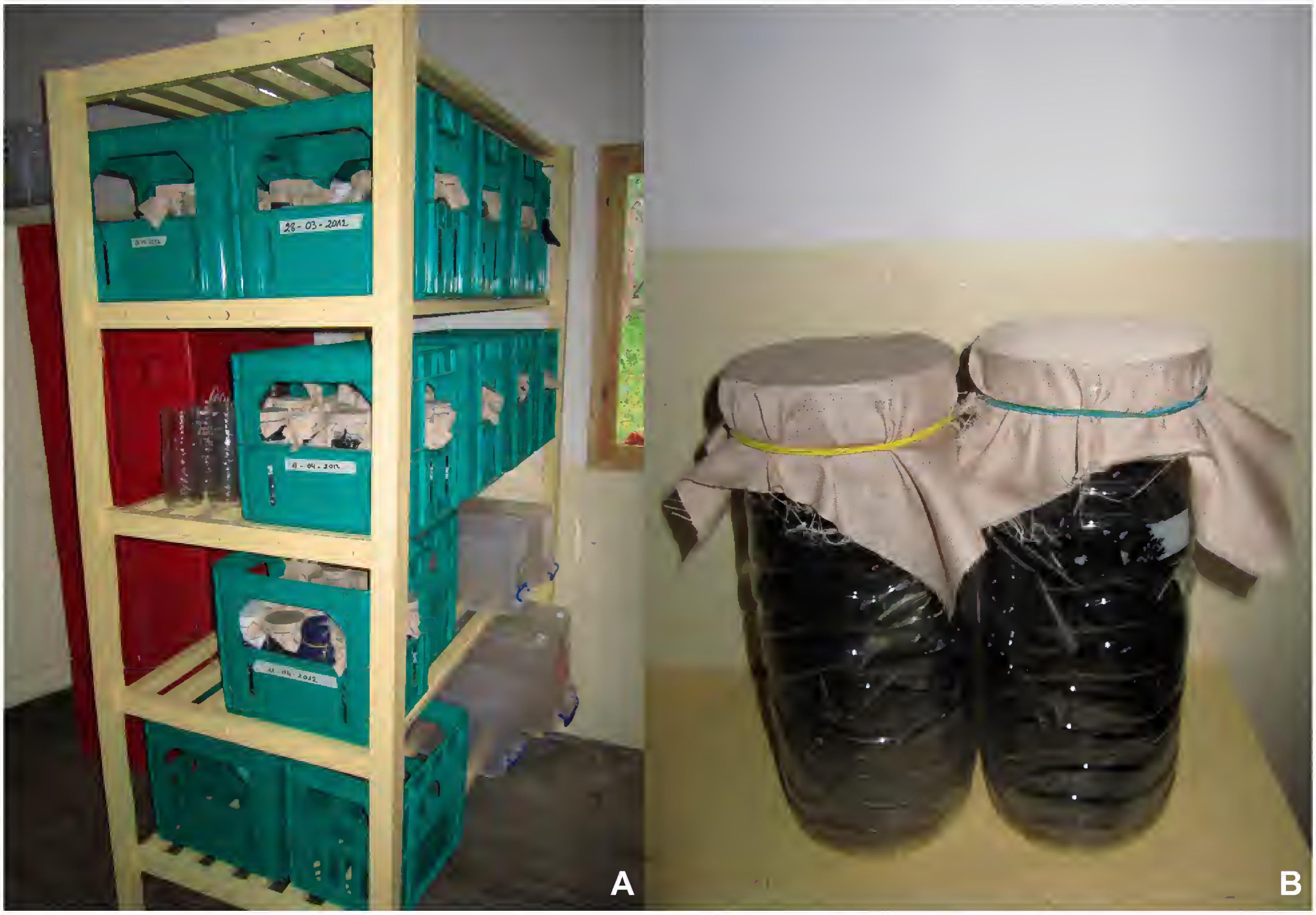


Figure 7. A) Fruit fly cultures on shelves at the facility. B) Fruit flies are cultured in discarded plastic water bottles collected in Andasibe. Fabric is secured in place, over the top with rubber bands, and strips of plastic bag are placed inside (above the media) on which the flies can deposit eggs.

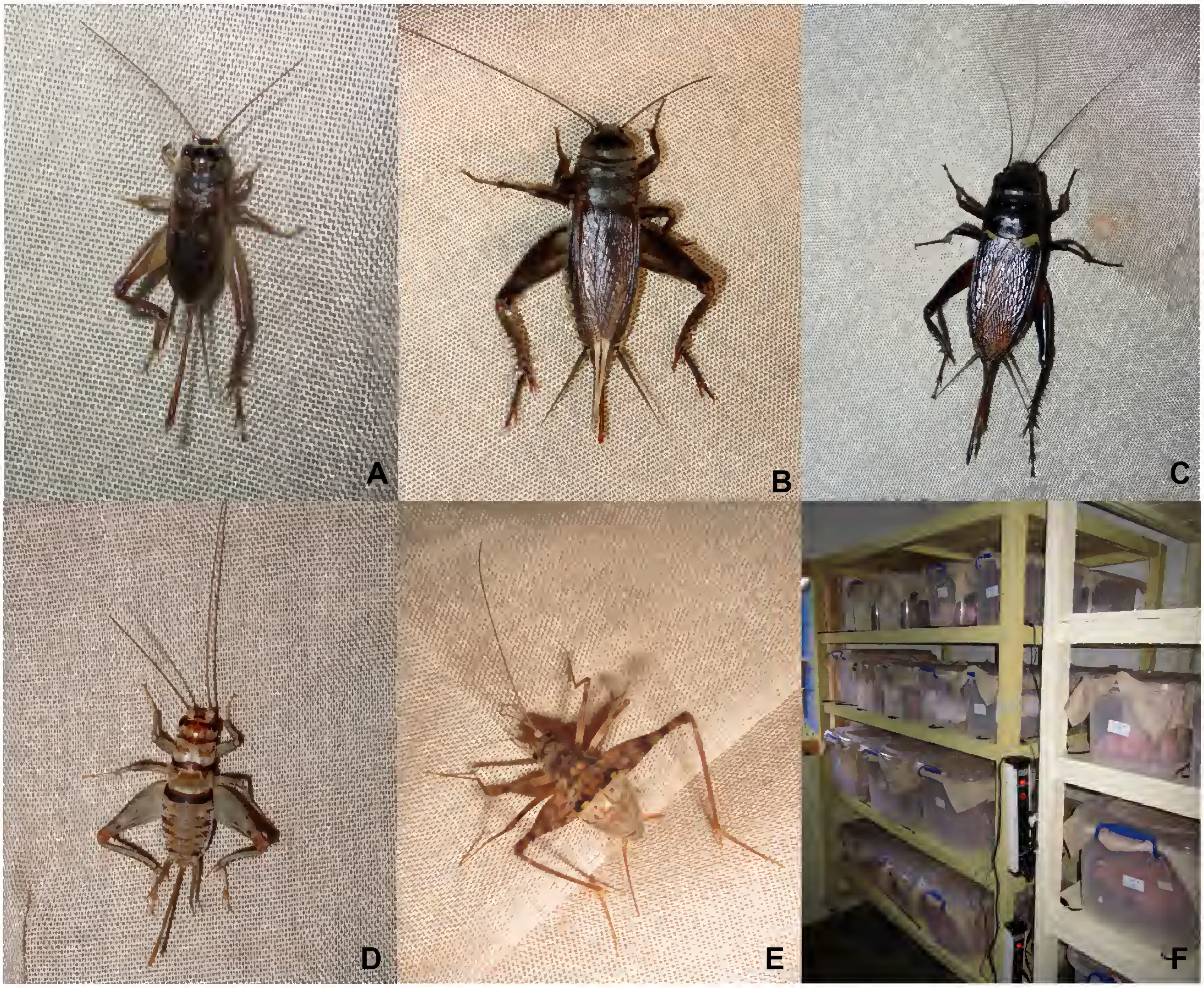


Figure 8. Locally-sourced crickets from Andasibe being bred at Mitsinjo's facility. A) Field cricket (*Modicogryllus* sp.). B) Large field cricket (*Modicogryllus* sp.). C) Large black cricket (*Gryllus* sp.). D) Tropical house cricket (*Gryllodes sigillatus*). E) Cave cricket (Rhaphidophoridae). F) Shelves with boxes housing field crickets and tropical house crickets.

market in the village, anticipating that their larvae could be used to vary the diet of small frog species. Unfortunately, they proved to reproduce very slowly, regardless of the media they were kept on (rice, pasta, flour, and peanuts were tried). Additionally, it was time consuming to harvest the larvae from the cultures. As a result, culturing this species was abandoned after one year.

In addition to isopods, cockroaches, and a small beetle species, Mitsinjo attempted to establish an earthworm culture in December 2010. More than 50 worms (species unknown) were collected from soil in Andasibe. Worms were placed into a box containing a mixture of soil and leaf litter. The box was kept outside in a cool location, and the moisture content of the substrate monitored regularly. Vegetable scraps were provided weekly as a food source. While most worms survived, no reproduction was noticed after more than four months and so the culture was discarded. It has recently been brought to our attention that vermiculture operations exist in Madagascar, and it is planned in the coming year to investigate the potential of culturing earthworms as a food source once again, starting with worms sourced from and using techniques developed by existing vermiculture operations in the area.

Frog husbandry research

The initial four species collected for training and husbandry research remained in good health throughout the first year, with two species (*Boophis pyrrhus* and *Mantidactylus betsileanus*) reproducing on multiple occasions. With no previously published accounts, this may represent the first captive breeding of these frog species. Detailed records of the conditions provided for these species will be disseminated in the future once the captive populations have been maintained for an extended period of time, and hypothesis-driven research has yielded significant results regarding their captive husbandry requirements.

As a first step towards conducting husbandry research on these species, tadpoles from the first clutch of eggs received from *M. betsileanus* were used in a preliminary training exercise to both help understand the optimal captive larval diet for this species and to train technicians how to conduct hypothesis-driven husbandry studies. Tadpoles were divided into three different aquaria, each one being fed a different diet, with observations made about the metamorphosed frogs which resulted from each group (Figure 9).

Although results from this first pilot-study were statistically inconclusive due to inconsistent data collection and lack of materials to measure and weigh the metamorphosed frogs, it was a beneficial exercise because it allowed technicians to learn how to formulate a hypothesis, collect data, and conduct their own research project. Mitsinjo plans to repeat this same study when *M. betsileanus* breed again, measuring all newly metamorphosed frogs with a caliper and recording all data regarding their development, including when each individual completes metamorphosis.

Conclusions and future outlook

Numerous authors and conservationists have discussed the pressing need to build capacity in Madagascar to manage captive populations of amphibians (Andreone 2006; Furrer 2008; Mendelson and Moore 2008). The development and implementation of the Mitsinjo breeding facility, which is the first project of its kind in Madagascar, is a step in the right direction. However, when considering the large number of individual captive frogs required to sustain an assurance population of even just one species for 10 years (as described by Schad 2007), and taking this into account alongside the exceptionally high frog species richness found in the Andasibe-area, it would be an enormous task to develop conservation breeding programs for more than a small fraction of the local frog species.

This fact highlights two important points. 1) It is imperative to develop additional capacity in Madagascar with other in-country organizations to manage captive assurance populations of amphibians, as well as to assess the specific conservation needs of species to prioritize those for breeding programs. 2) Captive breeding programs must have exit strategies and complement conservation activities which directly address the most pressing threats facing Madagascar's frogs, such as habitat protection, forest restoration, and environmental awareness and education campaigns.

The outlook for addressing these two points is promising. Notably an Amphibian Husbandry Workshop led by Durrell Wildlife Conservation Trust is scheduled to take place in Antananarivo during December 2012 to train additional organizations and institutions in Madagascar on frog husbandry techniques. This will help build further capacity within Malagasy organizations to manage captive populations of amphibians. Additionally, Mitsinjo is pursuing funding to develop an education and outreach center, which will display live frogs and associated informative graphics to help promote interest in and aware-

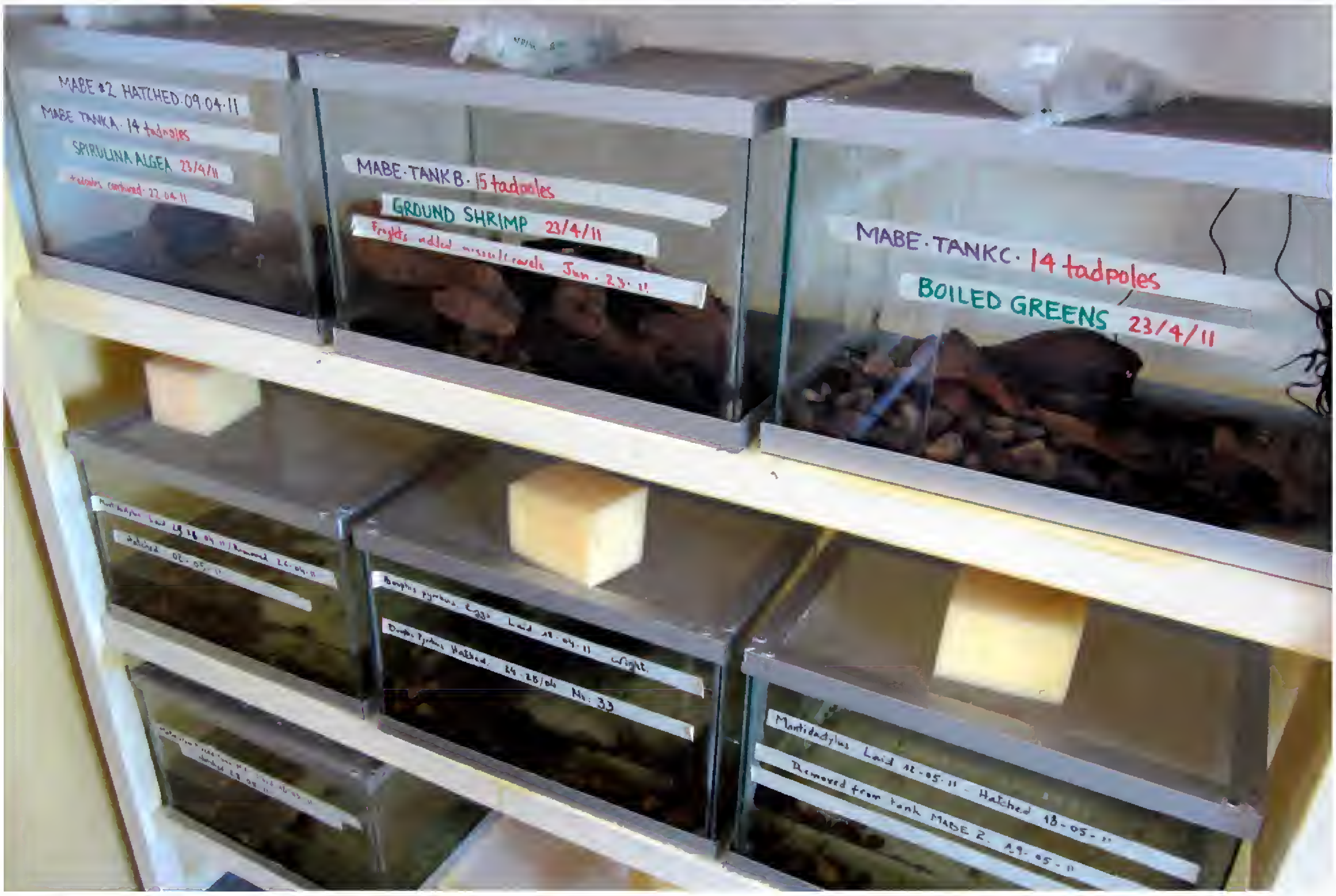


Figure 9. Pilot study and training exercise on the optimal larval diet for *Mantidactylus betsileanus*.

ness of the environment. This center will complement Mitsinjo's ongoing environmental education work in Andasibe.

Acknowledgments.—We are exceptionally grateful to the following organizations which have financially supported the development of the Mitsinjo captive breeding facility—Amphibian Ark Seed Grant, the Association of Zoos and Aquariums Conservation Endowment Fund, the Wildlife Conservation Society, Durrell Wildlife Conservation Trust, Cleveland Metroparks Zoo Africa Seed Grant, Tree Walkers International Amphibian Conservation Partnership Fund, Biopat, Understory Enterprises, American Frog Day, Conservation International, Toronto Zoo, and Woodland Park Zoo. We also wish to recognize Isabella Lau, Jaclyn Entringer, Matt Ward, Luke Harding, Janosch Heinermann, Stacey Boks, and Sebastian Wolf for their exceptional commitment spent volunteering time and expertise to the project, without which it would not be what it is today.

Literature cited

AmphibiaWeb: Information on amphibian biology and conservation. 2012. AmphibiaWeb, Berkeley, California. [Online]. http://amphibiaweb.org/ [Accessed: 15 May 2012].

Andreone F. 2006. The amphibian crisis and madagascar. In: *A Conservation Strategy for the Amphibians of Madagascar Conference: September 18-21, 2006.* Editor, Andreone F. Museo Regionale di Scienze Naturali, Torino, Italy. 2:44-58.

Andreone F, Cadle JE, Cox N, Glaw F, Nussbaum RA, Raxworthy CJ, Stuart NJ, Vallan D, Vences M. 2005. Species review of amphibian extinction risks in Madagascar: Conclusions from the Global Amphibian Assessment. *Conservation Biology* 19:1790-1802.

Andreone F, Carpenter AI, Copsey J, Crottini A, Garcia G, Jenkins RKB, Köhler J, Rabibisoa NHC, Randriamahazo H, Raxworthy CJ. 2012. Saving the diverse Malagasy amphibian fauna: Where are we four years after implementation of the *Sahonagasy Action Plan*? *Alytes* 29:44-58.

Andreone F, Carpenter AI, Cox N, du Preez L, Freeman K, Furrer S, García G, Glaw F, Glos J, Knox D, Köhler J, Mendelson III JR, Mercurio V, Mittermeier RA, Moore RD, Rabibisoa NHC, Randriamahazo H, Randrianasolo H, Rasomampionona Raminosoa N, Ravoahangimalala Ramilijaona O, Raxworthy CJ, Vallan D, Vences M, Vieites DR, Weldon C. 2008. The challenge of conserving amphibian megadiversity in Madagascar. *PLoS Biology* 6(5):e118.

Andreone F, Mercurio V, Mattioli F. 2006. Between environmental degradation and international pet trade: conservation strategies for the threatened amphibians of Madagascar. *Societa Italiana di Scienze Naturale e Museo Civico di Storia Naturale Milano* (Milano, Italy) 95(2):1-96.

Andreone F, Randriamahazo H. 2008. *Sahonagasy Action Plan*. Museo Regionale di Scienze Naturali, Conservation International, IUCN SSC Amphibian Specialist Group. Turin, Italy.

Dolch R. 2003. *Andasibe (Perinet): Are current efforts sufficient to protect Madagascar's biodiversity hotspot?* The Natural History of Madagascar. Editors, Goodman M, Benstead JP. Chicago and London: The University of Chicago Press, Chicago, Illinois, USA and London, United Kingdom. 1480-1485.

Furrrer S. 2008. Madagascar amphibian conservation in Zoo Zürich, Switzerland. In: *A Conservation Strategy for the Amphibians of Madagascar - Monografie XLV.* Editor, Andreone F. Museo Regionale di Scienze Naturali, Torino, Italy. 301-308.

Gagliardo R, Crump P, Griffith E, Mendelson J, Ross H, Zippel K. 2008. The principles of rapid response for amphibian conservation, using the programmes in Panama as an example. *International Zoo Yearbook* 42:125-135.

García G, Bock L, Earle S, Berridge R, Copsey J. 2008. Captive breeding as a tool for the conservation of Malagasy amphibians: how ready are we to respond to the need? In: *A Conservation Strategy for the Amphibians of Madagascar - Monografie XLV*. Editor, Andreone F. Museo Regionale di Scienze Naturali, Torino, Italy. 321-342.

Glaw F, Vences M. 2007. *A Field Guide to the Amphibians and Reptiles of Madagascar*. Third Edition. Vences & Glaw Verlag, Cologne, Germany. 496 p.

Griffiths R, Pavajeau L. 2008. Captive breeding, reintroduction, and the conservation of Amphibians. *Conservation Biology* 22(4):852-861.

IUCN 2011. IUCN Red List of Threatened Species. Version 2011.2. [Online]. Available: www.iucnredlist.org [Accessed: 16 April 2012].

Lötters S, Rödder D, Kielgast J, Glaw F. 2011. *Hotspots, conservation and diseases: Madagascar's megadiverse amphibians and the potential impact of chytridiomycosis in Biodiversity Hotspots.* Distribution and Protection of Conservation Priority Areas. Editors, Zachos FE, Habel JC. Springer, Hamburg, Germany. 255-274.

Mendelson JR III, Gagliardo RW, Andreone F, Buley KR, Coloma L, Garcia G, Gibson R, Lacy R, Lau MW, Murphy J, Pethiyagoda R, Pelican K, Pukazhenthi BS, Rabb G, Raffaelli J, Weissgold B, Wildt D, Feng X. 2007. Captive Programs. In: *Amphibian Conservation Action Plan.* Editors, Gascon C, Collins CJP, Moore RD, Church DR, McKay JE, Mendelson JR III. IUCN SSC Amphibian Specialist Group, Gland, Switzerland and Cambridge, United Kingdom. 36-37.

Mendelson JR III, Moore R. 2008. Amphibian conservation at the global, regional, and national level. In: *A*

Conservation Strategy for the Amphibians of Madagascar - Monografie XLV. Editor, Andreone F. Museo Regionale di Scienze Naturali, Torino, Italy. 15-20.

Pavajeau L, Zippel KC, Gibson R, Johnson K. 2008. Amphibian Ark and the 2008 Year of the Frog Campaign. *International Zoo Yearbook* 42:24-29.

Pessier A. 2008. Management of disease as a threat to amphibian conservation. *International Zoo Yearbook* 42:30-39.

Pessier AP, Mendelson JR (Editors). 2010. *A Manual for Control of Infectious Diseases in Amphibian Survival Assurance Colonies and Reintroduction Programs.* IUCN SSC Conservation Breeding Specialist Group, Apple Valley, Minnesota, USA.

Raxworthy CJ. 2008. Global warming and extinction risks for amphibians in Madagascar: A preliminary assessment of upslope displacement. In: *A Conservation Strategy for the Amphibians of Madagascar - Monografie XLV.* Editor, Andreone F. Museo Regionale di Scienze Naturali, Torino, Italy. 67-84.

Rabemananjara F, Andreone F, Rabibisoa N. 2011. Madagascar and chytrid news: Needed an urgent action and close collaboration between stakeholders. *Froglog* 97:33.

Schad K (Editor). 2007. Amphibian Population Management Guidelines. In: *Amphibian Ark Amphibian Population Management Workshop: December 10-11, 2007*; San Diego, California, USA. Amphibian Ark, www.amphibianark.org.

Weldon C, du Preez L, Vences M. 2008. Lack of detection of the amphibian chytrid fungus (*Batrachochytrium dendrobatidis*). In: *A Conservation Strategy for the Amphibians of Madagascar - Monografie XLV.* Editor, Andreone F. Museo Regionale di Scienze Naturali, Torino, Italy. 95-106.

Zippel K, Johnson K, Gagliardo R, Gibson R, McFadden M, Browne R, Martinez C, Townsend E. 2011. The Amphibian Ark: A global community for *ex situ* conservation of amphibians. *Herpetological Conservation and Biology* 6(3):340-352.

Received: 14 June 2012
Accepted: 01 July 2012
Published: 19 October 2012

Devin Edmonds has been keeping and breeding amphibians since early childhood and has authored three books about their captive husbandry. He completed his undergraduate studies at the University of Wisconsin-Madison in 2008 and holds a B.A. in Zoology. Since 2010, Devin has been living in Andasibe, Madagascar helping coordinate Mitsinjo's amphibian conservation activities.

Justin Claude Rakotoarisoa served as the Conservation Officer of Association Mitsinjo for more than eight years, and is currently the Lead Technician at the amphibian captive breeding facility discussed in this paper. He has helped carryout research on varied local taxa from around Andasibe, including conducting herpetofaunal inventories and studies on the weevil *Trachelophorus giraffa*.

Rainer Dolch, holding a Ph.D. in ecology from the University of Göttingen, has been working in Madagascar since 1992. As senior coordinator of the Malagasy conservation organization Association Mitsinjo, his interest and research has been focusing on Madagascar's threatened and endemic animal and plant species, stretching across a wide variety of taxa including amphibians.

Jennifer B. Pramuk is a curator at Woodland Park Zoo, Seattle Washington, USA. She has a background in amphibian and reptile reintroduction programs and in taxonomic herpetology and has published 27 peer-reviewed papers on related topics.

Ron Gagliardo has worked for Amphibian Ark since 2008 as their Training Officer. He is based out of Woodland Park Zoo, Seattle, Washington, USA and leads the development and implementation of AArk's Ex Situ Conservation Training Workshops which build capacity of individuals and institutions to conduct successful *ex situ* conservation programs for amphibian species. Trained as a chemist and with degrees in Botany from North Carolina State University, he maintains a deep interest in amphibians, reptiles, and their conservation.

Franco Andreone is curator of zoology and editor for scientific publications at the Museo Regionale di Scienze Naturali, Turin (Italy). As a member of several societies and editorial boards he is president of the International Society for the Study and Conservation of Amphibians (ISSCA) and co-chair of the IUCN SSC Amphibian Specialist Group for Madagascar.

Nirhy Rabibisoa is a researcher interested in the herpetofauna of Madagascar. He received his Ph.D. in 2008 dealing about the systematics, ecology, and biogeography of stream amphibians in Madagascar, with a focus on the subgenus *Ochthomantis*. Before becoming a Co-Chair of the ASG Madagascar, he coordinated and monitored the activities and programs related to ACSAM (A Conservation Strategy for the Amphibians of Madagascar) as the Amphibian Executive Secretary of IUCN at Conservation International Madagascar. Currently, he is a lecturer at the Mahajanga University and the Veterinary School in Antananarivo, Madagascar.

Falitiana Rabemananjara received his Ph.D. in 2008. He specialized on the molecular biology and phylogeography of amphibians at the University of Amsterdam, University of Braunchweig and Omaha Zoo. He is an ASG member and Coordinator of the Chytrid Emergency Cell in Madagascar. Currently, he is working on the conservation of the Critically Endangered amphibian species of the Ankaratra Massif.

Sahondra Rabesihanaka is head of fauna and flora management at the Ministry of Environment, Madagascar where she focuses on trade and the CITES convention. She holds a degree from the School of Agricultural Sciences at the University of Antananarivo.

Eric Robsomanitrandrasana holds a diploma in Forestry, Environment, and Development from the School of Agricultural Sciences at the University of Antananarivo, with initial training in biology, animal ecology, and conservation. Currently, he is responsible for wildlife at the Direction Générale des Forêts at the Ministry of Environment of Madagascar.

Copyright: © 2013 McFadden et al. This is an open-access article distributed under the terms of the Creative Commons Attribution–NonCommercial–NoDerivs 3.0 Unported License, which permits unrestricted use for non-commercial and education purposes only provided the original author and source are credited. The official publication credit source: *Amphibian & Reptile Conservation* at: amphibian-reptile-conservation.org.

Amphibian & Reptile Conservation 5(3): 70–87.

Captive management and breeding of the Critically Endangered Southern Corroboree Frog (*Pseudophryne corroboree*) (Moore 1953) at Taronga and Melbourne Zoos

[1]Michael McFadden, [2]Raelene Hobbs, [3]Gerry Marantelli, [4]Peter Harlow, [5]Chris Banks and [6]David Hunter

[1,4]*Taronga Conservation Society Australia, PO Box 20, Mosman, NSW, AUSTRALIA* [2,5]*Melbourne Zoo, PO Box 74, Parkville, Victoria, 3052, AUSTRALIA* [3]*Amphibian Research Centre, PO Box 1365, Pearcedale, Victoria, 3912, AUSTRALIA* [6]*NSW Office of Environment and Heritage, PO Box 733, Queanbeyan, NSW, 2620, AUSTRALIA*

Abstract.—The Southern Corroboree Frog *Pseudophryne corroboree* is a small myobatrachid frog from south-eastern Australia that has rapidly declined in recent decades largely due to disease, caused by infection with the amphibian chytrid fungus *Batrachochytrium dendrobatidis*. **As a key recovery effort to prevent the imminent extinction of this species, an *ex situ* captive breeding program has been established in a collaborative partnership between Australian zoological institutions and a state wildlife department. Despite initial difficulties, successful captive breeding protocols have been established. Key factors in achieving breeding in this species include providing an adequate pre-breeding cooling period for adult frogs, separation of sexes during the non-breeding period, allowing female mate-choice via the provision of numerous males per enclosure and permitting the females to attain significant mass prior to breeding. Difficulties were experienced with egg and larval mortality in early years, though these issues have since been largely resolved. To date, the success of captive breeding from 2010–2012 has permitted the reintroduction of 1,060 captive-produced eggs and an increasing captive population. size that will support conservation research and provide insurance against further declines.**

Keywords. *Pseudophryne corroboree*, captive breeding, husbandry, conservation, zoo, Anura, frog, Australia

Citation: McFadden M, Hobbs R, Marantelli G, Harlow P, Banks C, Hunter D. 2013. Captive management and breeding of the Critically Endangered Southern Corroboree Frog (*Pseudophryne corroboree*) (Moore 1953) at Taronga and Melbourne Zoos. *Amphibian & Reptile Conservation* 5(3): 70–87 (e72).

Correspondence. *Email:* [1]*mmcfadden@zoo.nsw.gov.au* (corresponding author) [2]*rhobbs@zoo.org.au* [3]*gerry@frogs.org.au* [4]*pharlow@zoo.nsw.gov.au* [5]*cbanks@zoo.org.au* [6]*david.hunter@environment.nsw.gov.au*

Introduction

Over the past five decades amphibians have been declining at a rate exceeding that of other terrestrial vertebrates (Stuart et al. 2004). A large proportion of these declines are due to the spread of the amphibian chytrid fungus (*Batrachochytrium dendrobatidis*), which causes the disease chytridiomycosis (Berger et al. 1998; Skerratt et al. 2007). There is currently no adequate management response that can reduce the population level impacts of this pathogen on susceptible species that continue to decline (Woodhams et al. 2011; McCallum 2012), and as such, the only way to prevent their complete extinction is to secure captive assurance colonies in quarantine facilities (Gagliardo et al. 2008). The large number of frog species in this situation necessitates a large scale response, and there has been a coordinated effort globally to increase the knowledge and resources required to achieve this (Zippel et al. 2011).Within Australia, 26 amphibian species have been identified as requiring *ex situ* intervention by the IUCN Global Amphibian Assessment, and State or Federal recovery plans (Gillespie et al. 2007). Of these species, the Southern Corroboree Frog (*Pseudophryne corroboree*) was considered the highest priority owing to its extremely precarious status in the wild (Gillespie et al. 2007). The Southern Corroboree Frog has suffered a rapid and catastrophic population decline since the mid-1980s (Osborne 1989; Osborne et al. 1999; Hunter et al. 2009b), with all the evidence implicating chytridiomycosis as the primary causal factor (Hunter et al. 2009c). It is now one of Australia's most threatened vertebrate species, with potentially fewer than 50 individuals remaining in the wild (Hunter et al. 2007), and no reproduction occurring in remnant wild populations in 2013. The species is listed as Critically Endangered by the IUCN (Hero et al. 2004). It is also listed as Critically Endangered nationally under the Environment Protection and Biodiversity Act 1999 and as Endangered under Schedule 1 of the NSW Threatened Species Conservation Act 1995.

The critically low abundance and continued decline of *P. corroboree* suggests that this species will become extinct in the wild in the very near future without immediate human intervention. Thus, persistence of the species in the wild will depend on the success of a captive breeding program combined with the targeted *in situ* release of captive-bred progeny, and ideally mitigation of the amphibian chytrid fungus. To enable this, a collaborative *ex situ* program has been established in partnership between NSW Office of Environment and Heritage (OEH) and four captive institutions. The primary aims of this captive program are to establish an insurance population and supply captive-bred progeny for reintroduction and conservation research.

Materials and Methods

Study Species

Pseudophryne corroboree is a small, robust terrestrial myobatrachid frog that is easily recognized by its unique and striking colouration. (Fig.1) The dorsal surface is boldly marked with black and yellow longitudinal stripes, while the ventral surface consists of black, yellow and white blotches. Adults reach a maximum length of between 25 and 30 mm (Cogger 2000). The species is restricted to Kosciuszko National Park in New South Wales (NSW), Australia, where it was historically known to occur across an area of 400 km^2 at altitudes of 1300–1760 metres (Osborne 1989). Within this range, its breeding habitat is largely associated with ephemeral pools within sphagnum bogs or wet tussock grasslands along watercourses (Hunter et al. 2009a).

Pseudophryne corroboree breeds annually from mid to late summer, with males creating small, terrestrial nest chambers. The females typically lay 16–38 large eggs, which measure eight mm in diameter when hydrated (Hunter et al. 2007), within the nest chamber. The male remains with the nest throughout the breeding period, often attracting clutches from multiple females within a single chamber. The eggs develop in these terrestrial nests through to hatching stage, at which point they enter diapause and await autumn rains to flood the nest. Flooding stimulates the eggs to hatch and the tadpoles to move into the main pool,where they become free swimming and feeding larvae. The tadpoles remain in the pool over the winter period and reach metamorphosis in late spring to early summer.

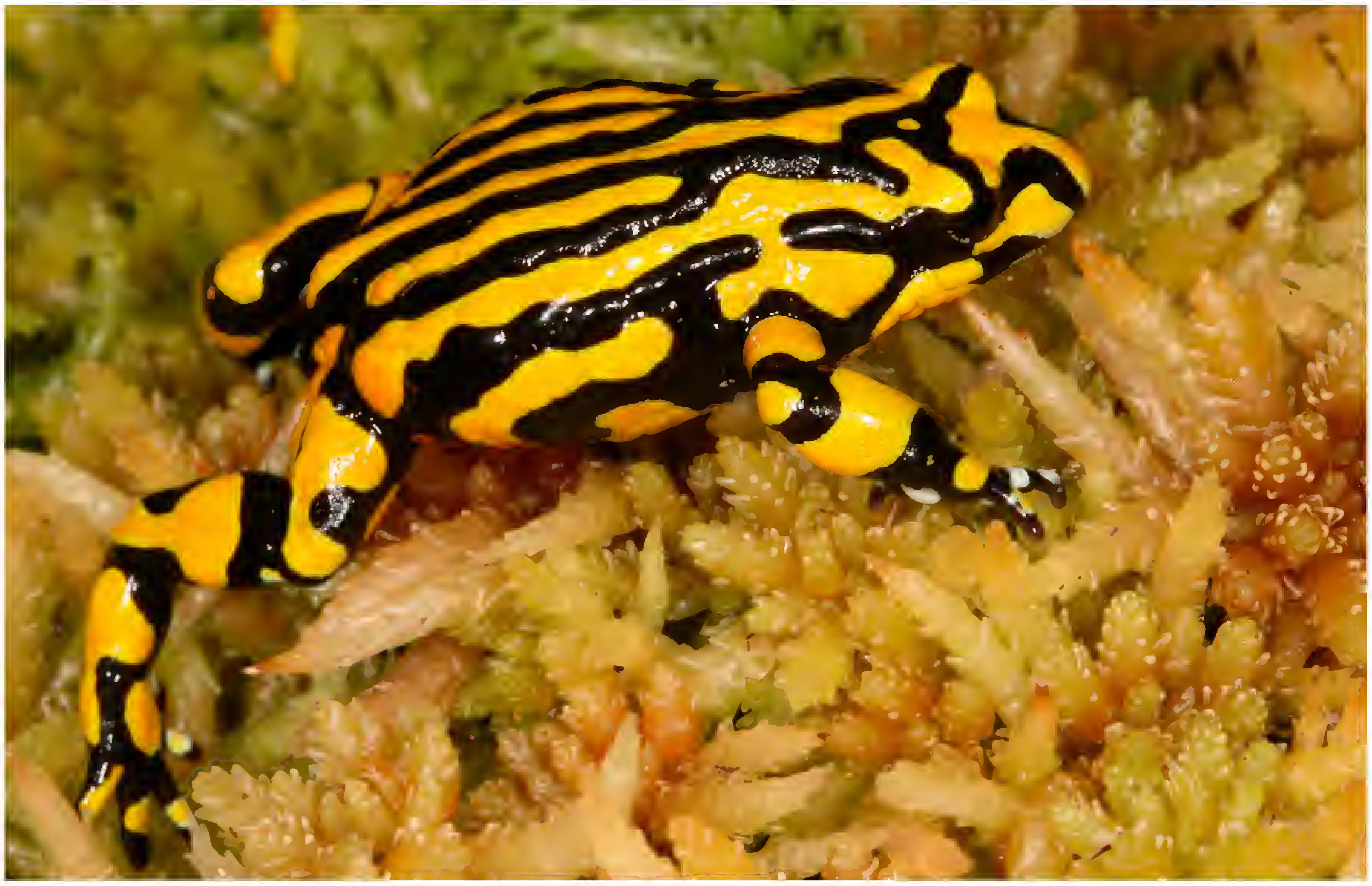


Fig. 1. Adult Southern Corroboree Frog.

Ex Situ Captive Management

The captive *P. corroboree* population is divided between four institutions: Taronga Zoo (TZ), Melbourne Zoo (MZ), Healesville Sanctuary (HS), and the Amphibian Research Centre (ARC). The captive program was initiated at the ARC in 1997, extending to MZ in 2001, TZ in 2006, and HS in 2007. Housing the frogs at a small number of dedicated institutions has dispersed the required resources and reduced the potential threat from disease, accident or natural disaster, yet still ensures tight control of biosecurity. The source of founders for the captive population has been from eggs collected in the wild between 1997 and 2012. This paper will focus on husbandry and breeding at TZ and MZ, which held 420 and 121 frogs respectively as of 1 November 2012. Many of the frogs contributing to the captive breeding outlined in this paper were initially reared to the juvenile or adult stage at the ARC before being transferred to TZ and MZ.

At both zoos, the *P. corroboree* populations are maintained in dedicated, isolated facilities equipped with refrigeration.(Fig. 2, Fig. 3)The refrigeration system is programmed to replicate the seasonal changes in the subalpine climate where this species occurs. The temperature control software is programmed with temperature alarms that also disable power to the facilities should the temperature become excessively high or low. Internal lighting within the facilities is controlled by light-sensitive switches set to simulate the local photoperiod. All water entering the facilities is filtered. To date, tadpoles have been successfully reared at TZ in water that has been filtered through a reverse osmosis (RO) unit alone; RO water reconstituted with trace elements and Sydney tap water that has been passed through a filtration system that constantly circulates water through five micron paper-pleated mechanical filters and activated carbon filters. Since 2010, the water at MZ is the municipal water supply that is recirculated through a sediment filter, a carbon filter, and a UV sterilizer. It then passes through an RO unit before entry into the facility.

High levels of biosecurity that comply with current recommendations (Pessier and Mendelson 2010) are maintained at both institutions. Facilities are serviced daily prior to contact with any other animal species, dedicated footwear is located within the facilities and must be worn upon entry and protective lab coats are worn. Disposable powder-free vinyl or latex gloves are kept within the facilities and are worn when handling any animal, enclosure or equipment.

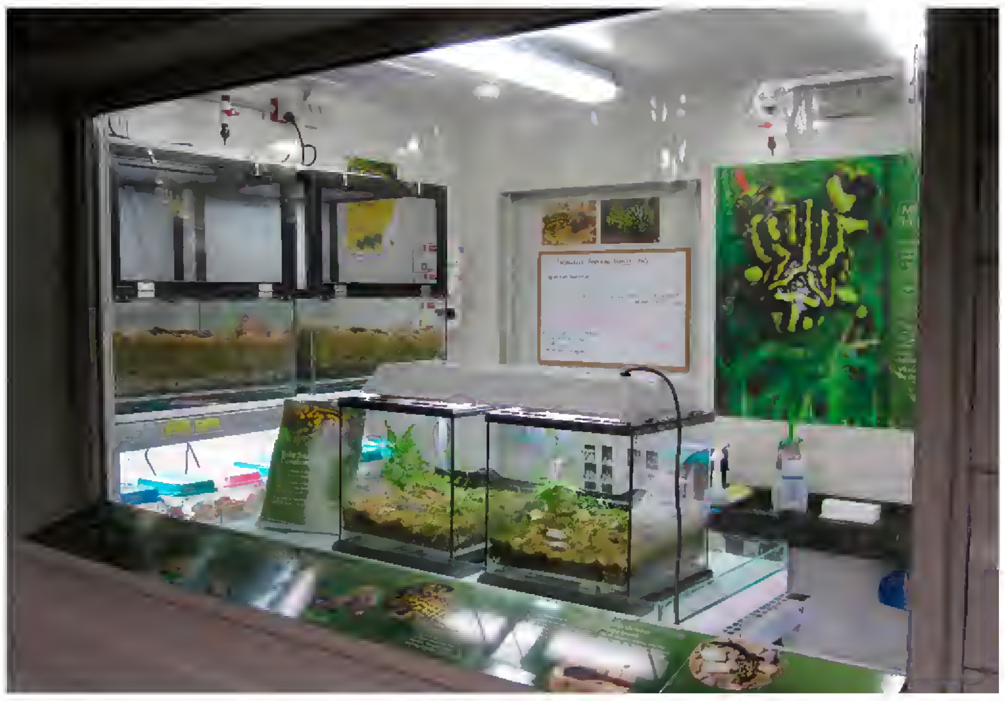


Fig. 2. Endangered amphibian complex at Melbourne Zoo.

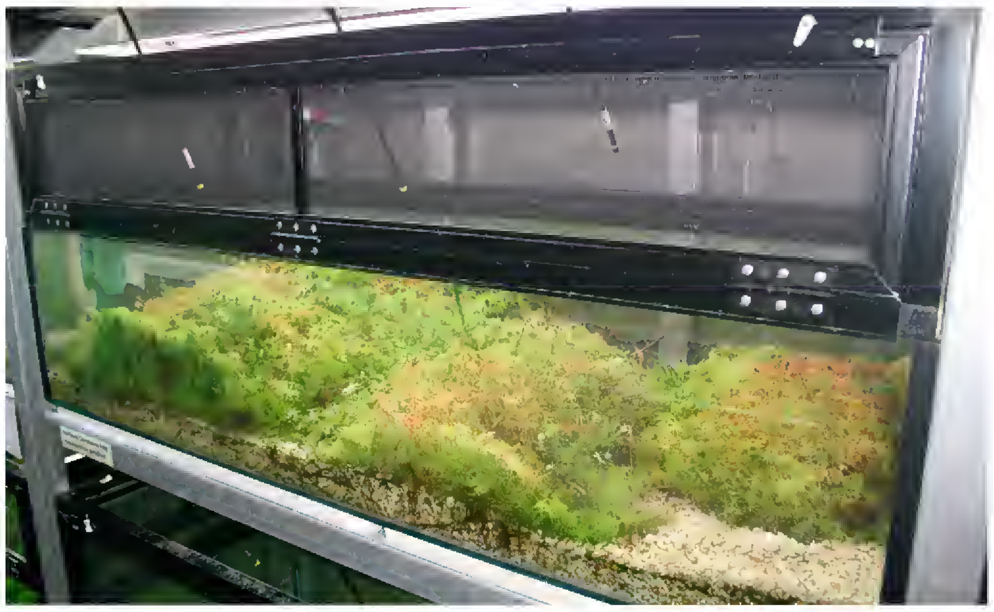


Fig. 3. Corroboree frog breeding enclosure at Taronga Zoo.

Captive Husbandry

The husbandry protocols described below apply at both institutions unless otherwise stated.

Housing – juveniles and adults in non-breeding season

Non-breeding adult frogs were housed in clear Hagen Pal Pen terraria of two sizes (27 × 17 × 20 cm and 33 × 19 × 24 cm). Each terrarium holds 4–6 frogs. The terrarium substrate is ~two cm of washed white aquarium gravel (particle size ~4 mm) that has been heat-sterilized at 200 °C for one hour. Three mm diameter holes were drilled in the base of the terrarium for drainage. Half of the floor area was either planted with live sphagnum or had a ~three cm layer of commercially-purchased dead, rehydrated sphagnum. At TZ, the moss was heated to 40 °C for 16 hours prior to use to ensure that any chytrid fungus zoospores were killed. At MZ, the moss was heated at 70 °C for 30 minutes, followed by 30 minutes at 40 °C. Ultraviolet light (UVB) was provided with Zoomed Repti-sun 10.0 fluorescent tubes situated 33 cm above the terrarium substrate. This typically provides UVB at between 20–30 µW / cm2 at the enclosure floor, as measured on a Solarmeter 6.2.

Diet

Frogs were reared primarily on a diet of 1–10 day old hatchling crickets (*Acheta domestica*). At TZ, they were fed twice per week from early December to late April (enclosure temperature 20–22 °C), once per week throughout November and from May to late August (14–18 °C) and not at all during September and October (5–10 °C). At MZ, frogs were fed 2–3 times per week from December to May (enclosure temperature at 25 °C and 15 °C, day and night respectively). Adult frogs were not fed during the cooler period which extends from June to November, when the temperature was below 10 °C. During each feed, the frogs were offered approximately 15–20 hatchling crickets each. The crickets were dusted with either Rep-Cal calcium or Herptivite multivitamin supplement, alternating between feeds. At MZ, frogs were also occasionally fed vestigial-winged fruit flies. Enclosure substrates were sprayed with water on the day after each feed to break down and wash away faecal waste and dead crickets.

Breeding Enclosures

At TZ, eight glass breeding tanks measured 135 × 55 × 55 cm high (including a 25 cm high fly-mesh hood with access doors). In 2010 and 2012, an additional glass tank measuring 120 × 70 × 65 cm (including a 35 cm high fly-mesh lid) was used. Each of the tanks had a base substrate of washed, heat-sterilised, 5–8 mm diameter white aquarium gravel. The tanks were planted with banks of live sphagnum moss slightly sunk into the gravel substrate. All moss was collected from within the direct breeding habitat of the species. In 2010, rather than live moss, one tank had commercially-purchased sphagnum moss installed around the outside of the tank to replicate the edge of a sphagnum pool.

At MZ, two different styles of enclosure have been used. A single tank was used in the 2006 and 2007 seasons. Two tanks were used in 2009 and 2010 seasons. These tanks mimicked a stream cross section with glass embankments on both sides. To replicate an alpine breeding environment, the tanks had a base of washed and heat-treated aquarium gravel, and substrate of commercially-purchased sphagnum moss (heat-treated and sterilized). These glass tanks measured 180 × 45.5 × 75 cm high (including fly-mesh hoods). The second tank had the same measurements except it had a lower height of 49 cm.

In mid-2010, the Endangered Amphibian Complex (EAC) at MZ was completed and commenced operation. This is a purpose-designed facility to simulate the temperatures found in the alpine areas of Australia. This room has two separate compartments with individual temperature controls. All of the *P. corroboree* were moved into the EAC in October 2010, just prior to the onset of the breeding season. There were four glass breeding enclosures; two measured 100 × 58 × 70 cm high (including 40 cm high fly- mesh hoods with access doors). The other two breeding tanks were smaller, measuring 65 × 58 × 70 cm high (including the same access door). Each tank had a base substrate of white aquarium gravel which had been washed and sterilized, and commercially-purchased sphagnum moss that had been heat-treated. The moss was placed into these breeding tanks to mimic the surrounding edges of an alpine bog and water was filled into the middle area of the pool.

Temperature Cycling

At TZ, immediately after the breeding season ends in early April, the adult frogs were placed in their non-breeding enclosures in single sex groups and maintained at 15 °C. In early September, the facility was cooled to 5 °C to replicate winter temperatures. The temperature was increased to 8–10 °C in mid-October, to 15 °C (with a 12 °C night setting) in early November and to 20 °C (with a 17 °C night setting) in mid-November. Once temperatures exceeded 15 °C, feeding of frogs resumed.

At MZ, the cooling regime has varied over the years due to a lack of facilities dedicated for ensuring these animals undergo a proper winter. During 2007, adult frogs were removed from their breeding enclosure and placed into plastic Pal Pen terraria for 64 days between November and January. These were cooled to 7–9 °C in a refrigerator during this period and the frogs were not offered food. These containers were watered very lightly to help simulate overwintering in drier habitats. After this period in the refrigerator they were then placed into breeding enclosures where the temperatures varied from 16–23 °C. Prior to the 2008–09 breeding season, 18 (3.5.10) adult frogs were placed into the refrigerator where temperatures ranged between 6–8 °C for seven weeks, and then moved into breeding tanks.

Prior to the onset of the 2010 breeding season, 18 adult frogs (same individuals as previous season) were placed into the fridge for 31 days at 6–8 °C. In 2011, all adult frogs were placed into the EAC rear compartment at 5–7 °C from 29 October to 04 December (males) and 20 December (females). Moving the frogs into the new facility at MZ has allowed the frogs to undergo a full year of temperature variation, similar to those maintained at TZ.

Tadpole Management

At TZ, tadpoles were generally maintained in 145 litre glass aquaria (122 × 70 × 17 cm deep), with between 20 and 120 tadpoles per aquarium. Up to 10 tadpoles have also been reared in 11 litre plastic aquaria (33 × 18 × 18 cm). At MZ the tadpole tanks have varied over the seasons, including within the breeding tanks and in 35 L of water in glass aquaria (75 × 29 × 30 cm). The current tadpole rearing tanks in the EAC (64 × 58 × 20 cm) have removable aluminium-framed fly-mesh divides in the centre, allowing two tanks to become four if required. These tanks hold approximately 50 litres.

Daily water changes of approximately 10% were conducted using an automated irrigation timer and spray system. Weekly water quality tests were undertaken to ensure water parameters are maintained within appropriate limits (ammonia – 0 ppm, nitrates – 0 ppm, pH 6.0–7.0, conductivity <15 µS/m).

Aquarium substrate was ~1 cm of pond silt collected from the bottom of natural pools within the species' habitat. Prior to use, the silt was heated to 40 °C for 24 hours to kill chytrid fungus zoospores (Johnson et al. 2003), a process which still allows algae to survive and grow. As well as feeding on algae, tadpoles were offered a diet of frozen endive twice per week and a 75:25 mixture of finely-powdered Sera Flora and Sera Sans fish flakes, three to four times per week. This tadpole diet has been utilized at TZ since 2007, with the heat-treated natural silt first added to tadpole rearing tanks at MZ during the 2009 breeding season. Prior to that, only endive was offered. In 2012, MZ also added finely crushed spirulina wafers.

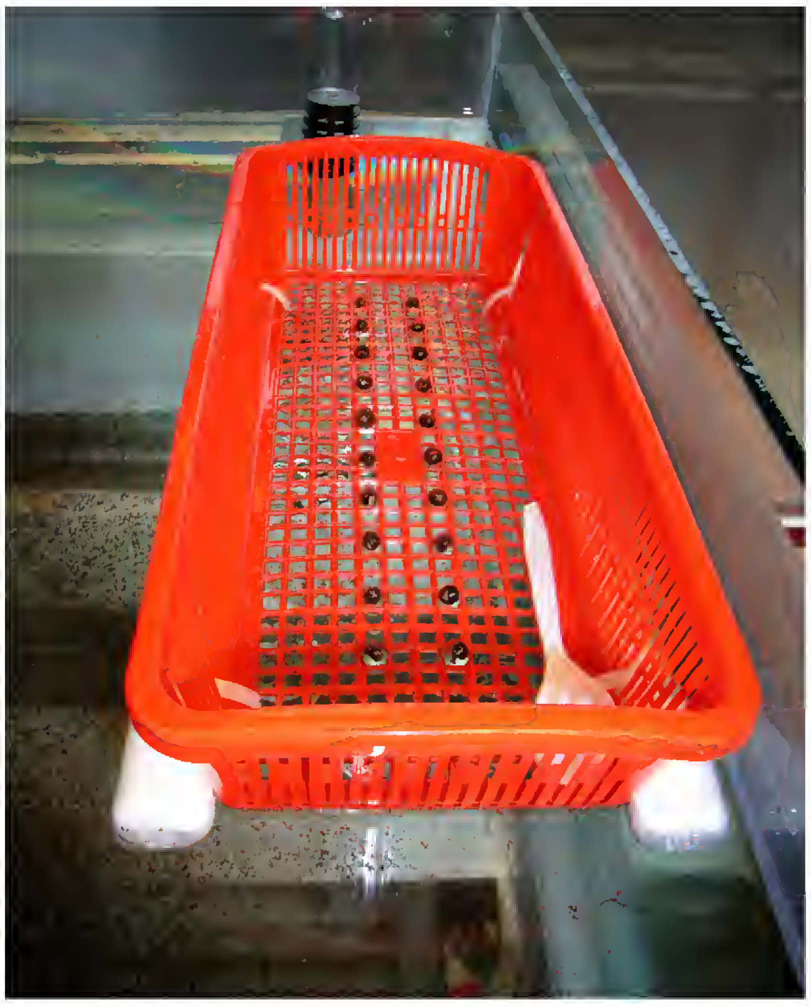


Fig. 4. Floating hatching tray on a tadpole rearing tank.

Results

Captive Breeding at Taronga Zoo

2010

Five males were placed in each of four breeding tanks from 28–31 December 2009, to allow them time to establish nests. Six female frogs were added to each tank on 26 January 2010. Five females in each tank were six years old, while one was four years old. The male frogs began calling on 23 January. One or two males were heard calling daily from each tank, with four frogs often heard calling from one of the tanks. Frogs often called in response to any sound (e.g., keeper entry into the facility), and could be stimulated to call at any time with a shout. In order to further stimulate calling activity, a cassette player with a 30-second continuous loop tape of a male calling was installed in both facilities on 31 January. The tape was set to come on for the first 15 minutes of each hour from 1800 to 2200 hours inclusive. The volume approximated a typical male calling in the facility, to be audible to the frogs in all tanks but not so loud as to dominate over the calling males. The calling frequency began to decrease from mid-March, ceasing on 26 March.

In late March, all tanks were searched, nests were located and the eggs removed. Six successful male nest sites were located, with two nests in each of the three tanks with live sphagnum moss. No nests were located in the tank with commercially-purchased sphagnum, despite the presence of calling males. To induce egg-laying, the three largest females from this tank were moved to another breeding tank on 28 March; two laid eggs in the following two weeks.

All nests were typically located between the sphagnum moss and the aquarium gravel. Only one nest was located inside a sphagnum clump. All nest sites were moist, but not saturated. The positioning of the eggs upon the gravel allowed for excellent drainage in the nest, but the moist sphagnum kept nest humidity at around 100%.

In total, 479 eggs were laid from a possible 24 mature females in 2010, suggesting that well over half of the females had laid eggs (Table 1). The numbers of eggs per nest varied from 36 to 130, indicating 1–4 clutches laid in each nest. Unfortunately, there was significant egg mortality, both while in the nest and following retrieval. Only 38% of eggs appeared live when removed from the nests, and 28% of the total survived eight weeks until Stage 27 (Gosner 1960), after which hatching can occur once eggs are inundated. Almost all mortality before and after removal from the nest occurred prior to Stage 14 (Gosner 1960). Eggs were kept at temperatures of 13.5–15 °C within the nest and while packed in live, moist sphagnum moss after removal, and all appeared to be well within the range of normal moisture levels observed in wild nests. It is important to note that infertile eggs could not be distinguished from embryos that died in early developmental stages, though the majority of the 72% failed eggs did appear fertile. A total of 134 embryos reached Stage 27 (hatching), with 47 of these released to Kosciuszko NP and the remainder retained for rearing.

2011

From 12–15 January, five males were placed in each of seven breeding tanks. On 22 February, five or six female frogs were added to each of six breeding tanks, with only one female added to the remaining tank. Calling activity was recorded from 30 January to 6 April. Between one and four frogs were recorded calling from each of the tanks. Calling was more consistent from the seven year old males, with at least one male strongly calling each day. Two of the four tanks with five year olds had weak or no calling on most days. To further stimulate calling behavior, call playback was again used from 22 February.

On 25 March, a total of 422 eggs were removed from six nests in the seven tanks (Table 1). Total number of eggs varied from 16 to 135 per nest, indicating clutches from one to five females in each nest. No eggs were laid in the tank containing only one female. There was a marked difference in productivity between the five and seven year olds, with older frogs laying more eggs. Based on the number of eggs laid, it appeared that over half of the seven year old females produced eggs. Additionally, embryo survival was 83%. The five year old females produced only two clutches of eggs (*n*=56) laid in nests, while three infertile clutches were scattered over the sphagnum moss. Within these two nests, embryo mortality was also higher than the seven year olds, but far less than in the previous year (Table 1). A total of 244 healthy embryos at hatching stage were released in Kosciuszko NP, while the remainder were retained at TZ.

2012

On 15 January, four to six males were added to each of eight breeding tanks. On 20 February, five or six female frogs were added to each tank. Three of the breeding tanks housed eight year old frogs, four housed six year old frogs, and the eighth tank housed six year old males and four year old females. Calling activity was recorded from 18 January to 08 April, with one or two males calling daily from each tank for most of this period. As calling behavior was more consistent in 2012, call playback was not utilized.

On 04 April, a total of 698 eggs were removed from 13 nests in seven tanks in the main breeding facility (Table 1). An additional 25 eggs were laid in a tank of males and females of mixed age in a second facility not detailed above. Number of eggs in each nest varied from 10–90, indicating one to three clutches being laid in each nest. Unlike 2011, there was no difference in the number of eggs produced between the two older cohorts of females, aged two years apart. Overall, 78% of embryos from these cohorts survived until hatching. However, four year old females showed lower fecundity, with only two clutches produced and 62% embryo viability until hatching. In 2012, 447 eggs at hatching stage were released and a small number were retained at TZ.

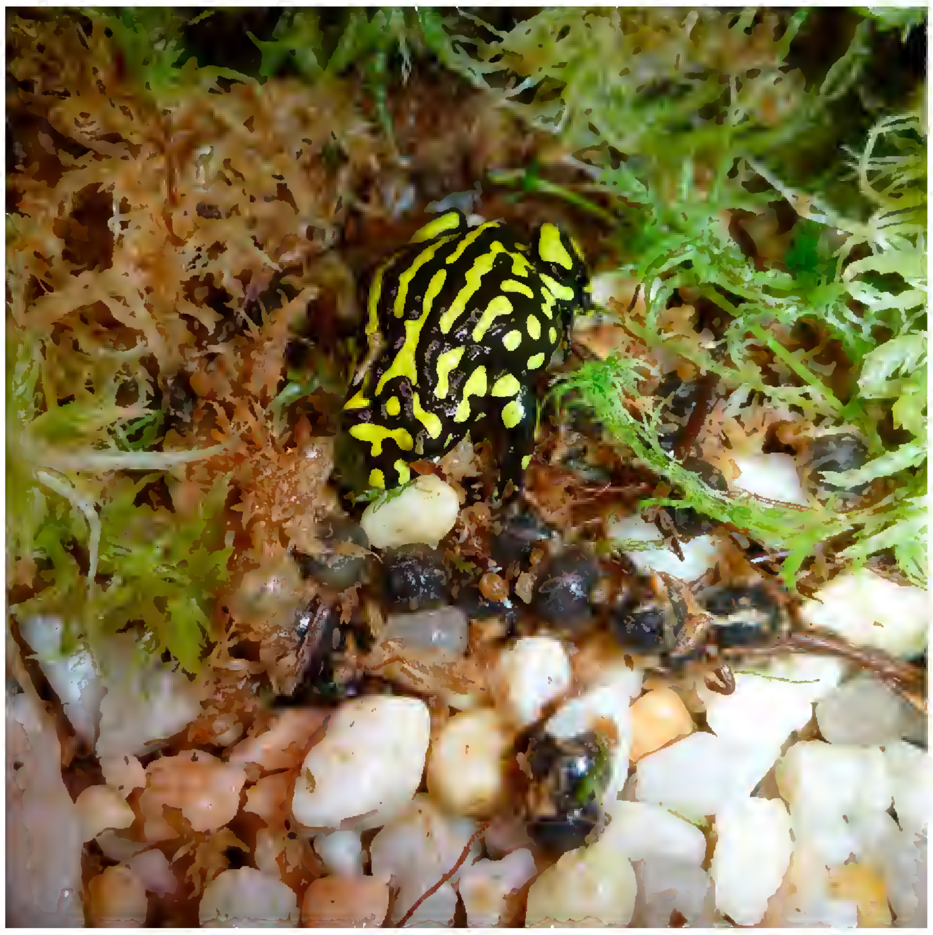


Fig. 5. Captive nest containing eggs.

Table 1. Breeding results for *P. corroboree* at Taronga Zoo in the 2010, 2011, and 2012 breeding seasons. All weights were taken just prior to breeding in January or February.

	2010	**2011**		**2012**		
No. of adult frogs used (♂.♀)	20.24	15.17	20.18	14.18	23.15	5.5
Age (years)	6	7	5	8	6	♀: 4 ♂: 6
Ave. female mass (g) (range)	2.9 (2.2–3.6)	3.06 (2.56–3.81)	2.85 (2.56–3.33)	2.83 (2.24–3.36)	2.93 (2.60–3.36)	2.83 (2.64–2.97)
Ave. male mass (g) (range)	1.8 (1.6–1.9)	2.17 (1.90–2.38)	1.94 (1.53–2.29)	1.76 (1.19–2.19)	1.91 (1.63–2.38)	1.88 (1.76–2.08)
No. of nests	6	4	2	6	6	1
No. of eggs produced	479	316	106	316	329	53
No. of eggs / total females	20.0	18.6	5.8	17.6	21.9	10.6
% mortality of eggs	72	17	34	26	19	38

Captive Breeding at Melbourne Zoo

2006 and 2007

Three to five adult frogs were maintained in a single breeding enclosure each year, with 42 and 46 eggs laid respectively (Table 2). Two tadpoles hatched within the enclosure's water area in the first year, with both subsequently metamorphosing within three months of hatching, but dying within 30 days. All of the eggs laid in 2007 were infertile.

2008

Ten additional four year old frogs were added to the breeding group but did not undergo a winter cooling prior to the breeding season as they arrived into the collection just prior. Upon completing quarantine protocols, these frogs were added to the group. Two males (from the new group of frogs) consistently called and attracted females. The original founder male died post-winter leading up to this season, therefore the existing breeding group total was reduced from five to four (all were known to be female by this stage). A total of 32 eggs were produced in what was thought to be two clutches. Two changes were implemented this season to address previous inadequate temperature control. First, eggs were removed from nests as soon as they were found, as high nest temperatures may not allow gaseous exchange, potentially asphyxiating the eggs. Second, the temperature at which eggs were held after removal from nests was reduced by placing them above cold, oxygenated water at 12 °C. Nest temperatures were 22 °C.

Many eggs died due to inadequate temperature control and only seven hatched. They were placed into a tank with water at 12 °C and all metamorphosed after 60–90 days. Three of the tadpoles presented with curvature of their tails. All metamorphs died 7–34 days post-metamorphosis and exhibited abnormal front limb emergence and mouth development. Post mortem examination of two frogs found bacterial and protozoan infections.

2009

Nineteen adult frogs were used for this breeding season, with a known sex ratio of 3.5.11. Six males were recorded calling from within nests. All males had constructed nests sites in and around the edges of the pond within sphagnum moss. Between 11 March and 14 April, 187 eggs were laid in the breeding tanks. Most eggs were removed from the nests immediately after being found and placed into sphagnum moss-filled containers on the surface of cold water at 8 °C. One clutch of 33 eggs was left in one nest, but there was no significance difference in egg mortality between the two rearing methods.

During May, the eggs were ready to hatch and were placed onto a floating, perforated plastic tray in a rearing tank where the water temperature was 12 °C.Water temperature was reduced to 5 °C between July-August and then gradually increased to 12-15 °C from November-December,giving the tadpoles a development period of 6-9 months.

Many eggs became cloudy and died quite early in development (Table 2).Some eggs developed a brown algal-like growth on the outer jelly layer, while others stopped developing and died in the egg. The outer casing of other eggs appeared “soft” and some tadpoles were underdeveloped and fell out of the egg membrane. Only 16 tadpoles hatched from the 187 eggs (8.5%) and 12 frogs metamorphosed. Five frogs died not long after metamorphosis, but seven were successfully raised. The metamorphs that died exhibited signs of hip dysplasia and deformed limbs, but this was not confirmed. These metamorphs were almost double the size of those from the previous seasons.

2010

After the cooling period, 20 adults were divided between two breeding enclosures. Seven males were recorded calling within nests. Male calls were recorded and three call types identified, i.e., advertisement, territorial and courtship. To enhance breeding suitability and egg production, females were moved between the two breeding enclosures to increase mate selection options. The females were weighed before being moved to more closely monitor weight fluctuations and identify females that had laid.

Once eggs were located, they were put into a fridge at 12–15 °C. Total number of eggs produced was 235. Eggs were laid between 13 March and 25 April. Egg mortality was again high at 77.5% with only 51 tadpoles hatching. After an average larval duration of six months, feeding on natural pond/bog silt and frozen endive, 43 frogs metamorphosed between October 2010 and January 2011, with post-metamorphic survival rate to one year old at 67.4% (29 frogs).

2011

The male frogs were placed in the four breeding enclosures (based on wild localities) within the EAC in December, while the females were kept separately and offered food *ad lib* for a further 16 days to allow males to establish nest sites. The three animals of unknown sex were grouped in with the females for this season. Despite the extra space and correct temperatures, only four males were heard calling, in two enclosures. After a number of weeks with little to no calling, frogs were removed from the two smaller tanks and placed into larger tanks, regardless of locality. After the movements, the number of males calling increased to six.

In total 119 eggs were laid in three clutches (average 39.6 eggs/clutch). Egg mortality was still high at 70%. These eggs produced 36 tadpoles and subsequently 33 metamorphs (91.6% larval survival rate). The post-metamorphosis survivorship was 100% until one year of age.

2012

On 28 August 2011, all adult frogs, including those whose gender was unknown, were removed from two breeding tanks and placed in plastic tanks for the remainder of their overwintering period. The males were cooled until 4 December (98 days) at temperatures varying from 5–12 °C. They were then placed into the breeding enclosures, with five males in each enclosure. Females were maintained at the above temperatures until 18 December (112 days). They continued to be kept separately from the males until the latter had started to call and had constructed nest sites. Females were placed into breeding tanks on 26 February (70 days after finishing overwintering period). Male frogs were not moved between enclosures due to nest establishment, but females were again moved to enhance mate choice options and compatibility, and likely breeding success. There were five or six female frogs in each enclosure at any time. Eggs were laid between 17 March and 17 April 2012, with a total of 556 eggs produced. These were likely to be from 17 clutches, with average female fecundity of 46.33 eggs (if laid by 12 known females) or 39.71, if the two frogs of unknown sex were also females that contributed to breeding. Three clutches of eggs were retained at MZ (total of 68 eggs) with a 28.4% egg mortality and 100% post-metamorphosis survival rate to the time of publishing, from 46 metamorphs produced. Larval hatching data were not collated this season as all eggs were hatched via assistance from keepers. All remaining 322 eggs produced this season were transferred to Kosciuszko NP for wild release.

Eggs

Once removed from the nest, eggs were packed in moist, live sphagnum moss in round plastic disposable food containers (12 × 10.5 cm high) with a lid on, air holes around the sides, and drainage holes in the base. The eggs were kept moist by lightly misting the moss with RO water every 10–14 days. Once the tadpoles reached about Stage 27 (Gosner 1960; Anstis 2002), the eggs were inundated in the tadpole rearing tank, allowing them to hatch and swim off. An alternative method used was to place the fully developed eggs on a floating, perforated plastic tray in the tadpole rearing tank, allowing the lower 1/3 of the egg to contact the surface of the water (Figure 9). This prevented eggs from desiccating, while allowing them to be easily inspected and the tadpole to hatch and swim away when fully developed. At TZ, the eggs began to hatch at five weeks if kept at 18 °C, but could take over six months if the eggs were kept between 5–10 °C. At MZ, between 2010 and 2012, eggs hatched between 74–95 days (10.5–13.5 weeks) at 13–23 °C. In the previous breeding seasons at MZ, eggs hatched quite early, at an earlier Gosner stage, resulting in high larval mortality.

Tadpoles

At TZ, the period of larval duration was usually four and a half to six months at 14–18 °C, including a seven to ten week period of over-winter cooling at 5 °C. Larval duration is as short as seven weeks at 18 °C, but the metamorphs emerged at a much smaller size. From 2007 to 2010, TZ had 372 frogs successfully metamorphose from 431 tadpoles (86% survival).

At MZ, larval duration varied from seven weeks to eight months. Prior to 2010, larval or early juvenile mortality was very high, with few surviving substantially past metamorphosis. Since 2010, with the implementation of a winter cooling during the larval period and the addition of a silt substrate, tadpole and metamorph survival increased significantly. The larval period now averages 213 days at temperatures varying seasonally from 5–23 °C throughout the six to nine month period.

Rearing Juveniles

At TZ, a subset of 17 frogs was weighed and measured at metamorphosis in 2009: length ranged from 11.3–13.8 mm (mean 12.5 mm) and weight from 0.20–0.38 g (mean 0.28 g). They were housed in identical conditions to the adult frogs, and readily accepted day old crickets. Post-metamorphic survival in captive *P. corroboree* is typically quite high with less than 5% mortality observed in their first year at TZ, from cohorts between 2007 and 2011.

At both zoos, male frogs can be heard calling at two years of age, though most males matured at three to four years. Earliest female breeding at TZ was from a single three year old frog from 19 individual females in this age group.

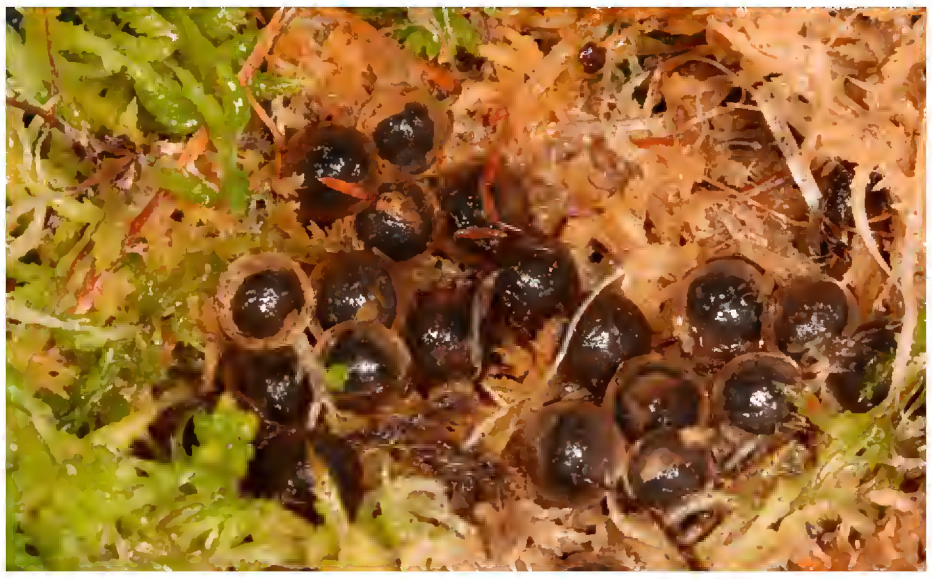


Fig. 6. Southern Corroboree Frog eggs.

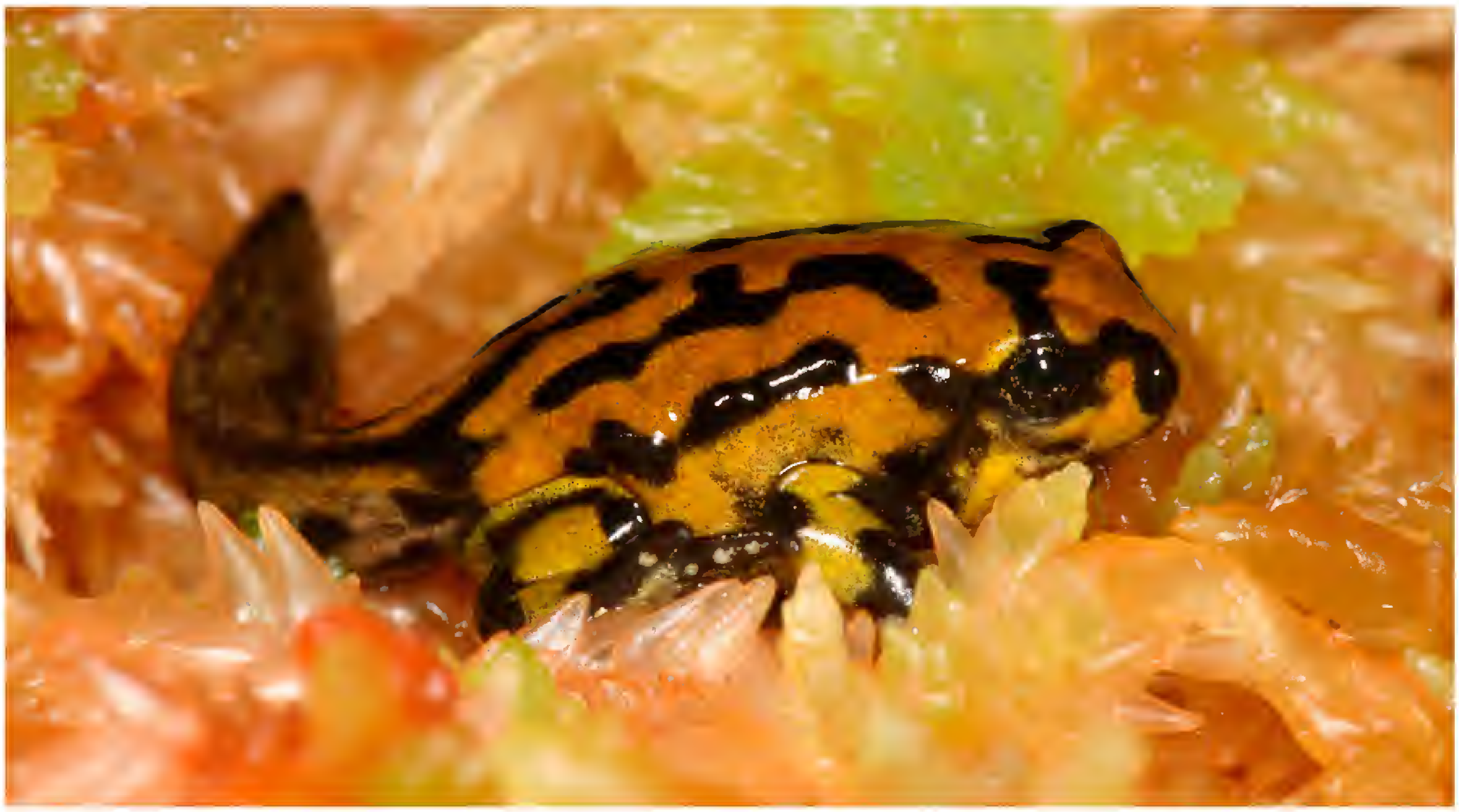


Fig. 7. Metamorphosing Southern Corroboree Frog.

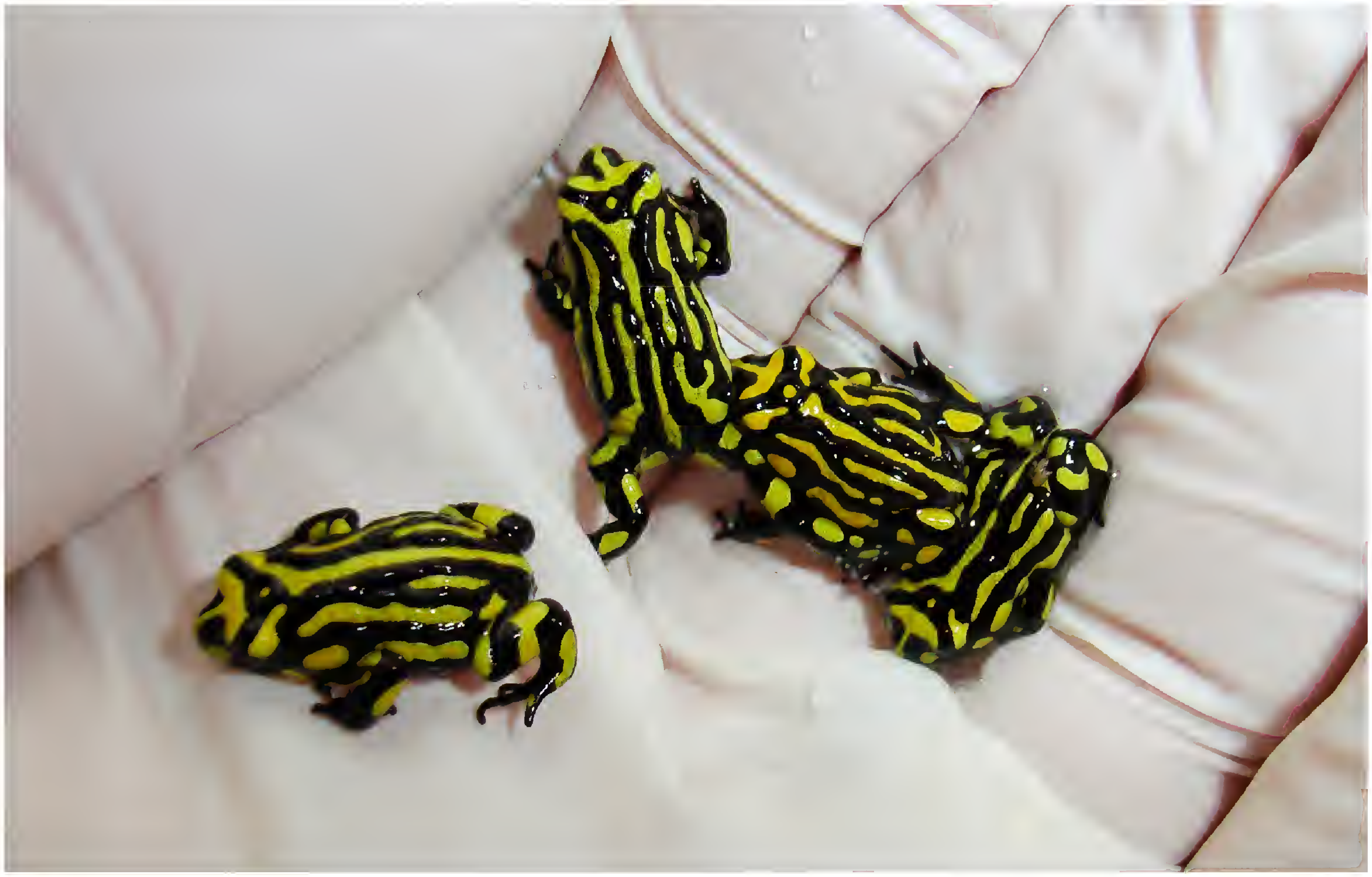


Fig. 8. Southern Corroboree Frog metamorphs.

Table 2. Breeding results for *P. corroboree* at Melbourne Zoo from 2006 to 2012. All weights were taken just prior to breeding in February or March.

	2006	2007	2008	2009	2010	2011	2012
No. of adult frogs used (♂.♀.unknown)	1.2	1.2.2	2.4.8	6.6.7	7.7.6	10.11.3	10.12.2
Ave. female mass (g) (range)	—	—	—	2.46 (1.85–2.84)	3.17 (2.79–3.72)	3.42 (2.74–3.97)	3.58 (2.92–4.63)
No clutches laid	1-2	3	2	11	12	3	17
No. of nests	—	—	2	6+	7	3	12
Eggs laid	42	46	32	187	235	119	556
Average clutch size	21	15.3	16	17	19.58	39.6	46.33
% mortality of eggs	95.3	100	78.2	91.5	77.5	69.8	27.1

Discussion

The *ex situ* conservation program for *P. corroboree* is an important Australian captive breeding program due to the iconic nature of the species and the critical status of wild populations. Refinement of husbandry techniques over the last seven years has led to improved breeding success and has allowed for the release of captive-bred eggs into the wild for experimental reintroductions. The likely reasons for our increased captive breeding success include provision of an adequate winter cooling period, the timing of introduction for breeding, placing multiple males in breeding tanks, and the correct age and body weight of frogs (especially females).

Reproductive Behavior

Pseudophryne corroboree is a sub-alpine species, with wild frogs brumating at temperatures below 5 °C under a layer of snow between June and August (Green and Osborne 2012). The frogs at both institutions were exposed to an overwintering period at 5 °C, though this period was shorter and later than in the wild in order to allow the females to increase weight between breeding seasons. We assume that a winter cooling period is important for reproduction in this species, but we did not investigate the critical overwintering temperature or minimum time required to permit reproduction. In the wild, the mean daily maximum temperature in *P. corroboree* habitat is below 5 °C for three months of the year (Bureau of Meteorology 2012).

Providing females with mate-choice by establishing multiple males in each breeding tank may have also contributed to the increase in reproductive success. Within each breeding tank, not all males established nests or called and there was a marked difference between the success of individual males, suggesting that females were demonstrating mate choice. Both zoos have also had gravid females that did not lay eggs in their breeding tanks by the end of the breeding season, but laid eggs shortly after they were moved to another tank. This suggests that they may not have been satisfied with the males or nest sites within the original tank. Female mate choice is quite widespread among anurans, with choice determined by a number of possible factors, including call frequency, male body size or male territory (Gerhardt and Huber 2002; Sullivan et al. 1995). Although mate choice is apparent in captive *P. corroboree,* it is not clear which characteristics females utilize to assess mate quality.

The separation of sexes outside the breeding season and the timing of their introduction to breeding tanks may be additional factors contributing to breeding success. The establishment of males in breeding tanks prior to the introduction of females allowed nest construction and commencement of calling activity before females were present, which would be consistent with the timing of these events in the wild. This also allowed the females to be fed more intensively in smaller terraria while their eggs were developing. Introducing the sexes once the eggs were developed, and the males were calling strongly, appeared to initiate almost immediate reproductive behavior in the captive *P. corroboree*.

Size and age at reproduction may have dictated the level of breeding success. Under wild conditions, age to first reproduction in males is typically four years, with a small proportion reaching sexual maturity at three years (Hunter 2000). It is suspected females may take four to five years. This species may live in the wild to at least nine years (Hunter 2000). Although frogs reached maturity in the zoos at a similar age, reproductive success was greatly reduced in younger frogs. At TZ, frogs at five years of age or below had limited breeding success, with significantly fewer males calling and females laying eggs. From six years of age onwards, breeding success greatly increased. Size was also important as females at TZ below 2.5 grams did not produce eggs, and successful spawning was higher in females over three grams. At MZ, females also began to mature at four years of age, with many requiring a further one to two years before reproducing (based on egg numbers and survival to hatching). Males at MZ appeared to attain maximum breeding success at seven years of age.

At MZ, it is possible that some females showed either egg-partitioning or double-clutching from the 2009 season onwards. The strongest indication of this was in 2012 when a maximum of 14 females were present (12 known females and two additional unsexed frogs) and eggs were laid in 17 whole, or partial, clutches. The large number of eggs per female is also consistent with this possibility as there was an average 39.7 eggs per female if all 14 females laid eggs. Under natural conditions, a female typically lays 16–38 eggs (Pengilley 1973).

Although double-clutching is not likely in the wild, it could possibly occur in captivity due to the availability of resources. Double clutching has been recorded previously in a captive *Pseudophryne australis*, though this species breeds continuously throughout the year after rainfall (Thumm and Mahony 2002), rather than seasonally in *P. corroboree*. It is also possible that females demonstrated as polyandry, laying eggs in more than one nest. Sequential polyandry has been described in another frog from this genus, *P. bibroni,* with females partitioning their eggs between the nests of up to eight males (Byrne and Keogh 2009). In this scenario, the large average clutch size could be explained by the above average mass of females allowing for greater reproductive investment resulting in larger clutches (Wells 2007; Jorgensen 1992; Kaplan 1987). Breeding females at MZ were much larger than wild females, with those producing larger clutches weighing significantly more than wild frogs.

Egg/Embryo Mortality

High mortality of captive-laid eggs and embryos has been a significant problem in this program (>65 % mortality at MZ between 2006 and 2011; 72 % at TZ in 2010). The high egg mortality seems to have been mostly resolved over the last two years, though the reasons for this are not fully understood. In the wild, excluding during drought, early embryo mortality is quite low at less than 15% (Pengilley 1992; Hunter et al. 1999). Moisture and pH characteristics of nests in captivity closely resembled those in the wild, and although nest temperatures in captivity at MZ often exceeded those in the wild, this was not the case at TZ in 2010. The fact that the same TZ breeding tank assemblages in which there was high egg/embryo mortality in 2010 (72%) experienced only 17% mortality in the following season suggests that nest substrate was not the cause of earlier mortality. Temperature may have influenced embryo mortality at MZ prior to 2012, as nest temperatures were frequently higher than those experienced in the wild. Maintaining eggs at temperatures higher than the optimum range has been demonstrated to cause embryo mortality in anurans (Goncharov et al. 1989), including other species of *Pseudophryne* (Seymour et al. 1991).

Other possibilities considered were the husbandry of embryos once removed from the nest and inadequate nutrition of females which might result in eggs with smaller yolk supplies, or other causes of inviability. It is noteworthy that during 2008 and 2009, approximately 2,600 wild-laid embryos at various stages of development were collected and reared at TZ for three months before return to the wild. Under conditions identical to those used for captive-laid embryos, mortality was only 11%, suggesting that husbandry of the eggs post-removal from the nest was not a contributing factor. Small trials were carried out at TZ in 2011 to test for the effect of diet and supplementation on embryo mortality. Due to the subsequent low egg mortality across all treatments, the results were inconclusive, and thus the factors responsible for the high egg/embryo mortality in the early years of the program remain unclear.

Larval Mortality

Tadpoles produced by the breeding program at MZ between 2006 and 2009 showed reduced vigour, high mortality, and produced smaller frogs at metamorphosis. Two factors may have contributed to this outcome. The first is that high water temperatures caused the larval period to be reduced to two to three months and there was no simulated overwinter cooling period. Current practice with inclusion of an overwintering interval has increased the larval life-span to six to nine months at MZ, or five to six months at TZ, approximating the wild larval duration. It seems likely that a larval duration of at least 140 days may be important for development of robust larvae and metamorph frogs, and high rates of metamorphosis.

The other significant factor was probably larval nutrition. From the 2010 season onwards, heat-treated silt from a Kosciuszko NP breeding site was added to the rearing tanks, and there was an immediate increase in larval viability from that year. The likely importance of both factors are supported by results at TZ from 2007 to 2011, where tadpoles have always undergone an overwinter cooling period and have had access to natural silt, as well as endive and fish flake. This resulted in 86% survival of larvae to metamorphosis at TZ during this period and high survivorship of metamorphs.

Conclusion

In view of its continued decline toward extinction, the survival of *P. corroboree* depends on the success of *ex situ* conservation measures. The development of successful captive-breeding protocols for this species has allowed the *ex situ* program to begin to offer *in situ* support, with the return of 738 (TZ) and 322 (MZ) captive-bred embryos to the wild between 2010 and 2012 (Hunter et al. 2010). Since the bulk of the captive population is now made up of immature frogs, the rate of production of embryos can be expected to rise over the next few years, ensuring the continued viability of the captive breeding population and greater capacity to undertake reintroductions back to the wild.

The more general lesson to be drawn from this program is that the development of reliable captive-breeding programs for species whose life history is unusual and/or not well known may invariably be both slow and highly demanding of skills and resources. It needs to be recognized that appropriate husbandry skills and breeding protocols should be in place before wild populations are reduced to critically low levels. The Sharp-snouted Day Frog (*Taudactylus acutirostris*) is a prime example of this: the delayed approval from the state government agency to establish a captive colony prior to population crashes and the combination of chytrid fungus infection (not recognized before 1998) and lack of experience in the appropriate husbandry of this genus led to the failure of a last-minute attempt to establish a captive population in 1993, and the species is now presumed extinct (Banks and McCracken 2002; Schloegel et al. 2005). Gagliardo et al. (2008) and Mendelson (2011) provide discussions of comparable instances of rescue operations for Critically Endangered amphibians in Central America. Thus, the development of husbandry protocols, for taxa with unusual biology or species in early decline, should be a conservation priority for *ex situ* institutions.

Acknowledgments.—We thank the Herpetology Department keepers at both TZ and MZ for assisting in the husbandry of the frogs. We also thank Angus Martin, Lee Skerratt, Laura Grogan, and Jon Kolby for helpful comments on the manuscript.

Literature Cited

Anstis M. 2002. *Tadpoles of South-eastern Australia: A guide with keys*. New Holland (Australia) Pty Ltd, Sydney, Australia.

Banks C, McCracken H. 2002. Captive management and pathology of sharp snouted day frogs, *Taudactylus acutirostris*, at Melbourne and Taronga Zoos. Pp. 94–102 In: *Frogs in the Community.*Proceeding of the Brisbane Symposium of the Queensland Frog Society, East Brisbane. Editor Nattrass AEO. Queensland Frog Society, Brisbane, Australia.

Berger L, Speare R, Daszak P, Green DE, Cunningham AA, Goggin CL, Slocombe R, Ragan MA, Hyatt AD, McDonald KR, Hines HB, Lips KR, Marantelli G, Parkes H. 1998. Chytridiomycosis causes amphibian mortality associated with population declines in the rainforests of Australia and Central America. *Proceedings of the National Academy of Sciences of the United States of America* 95: 9031–9036.

Bureau of Meteorology. Available: http://www.bom.gov.au/climate/averages/tables/cw_072091.shtml [Accessed: 05 December 2012].

Byrne PG, Keogh JS. 2009. Extreme sequential polyandry insures against nest failure in a frog. *Proceedings of the Royal Society of London, Series B, Biological Sciences* 276(1654): 115–120.

Cogger H. 2000. *Reptiles and Amphibians of Australia* (6th edition). New Holland (Australia) Pty Ltd, Sydney, Australia.

Gagliardo R, Crump P, Griffith E, Mendelson J, Ross H, Zippel K. 2008. The principles of rapid response for amphibian conservation, using the programs in Panama as an example. *International Zoo Yearbook* 42: 125–135.

Gerhardt HC, Huber F. 2002. *Acoustic Communication in Insects and Frogs: common problems and diverse solutions*. University of Chicago Press, Chicago, Illinois, USA.

Gillespie G, Traher R, Banks C. 2007. *ARAZPA Amphibian Action Plan*. ARAZPA, Syndey, Australia.

Goncharov BF, Shubravy OI, Serbinova IA, Uteshev VK. 1989. The USSR programme for breeding amphibians including rare and endangered species. *International Zoo Yearbook* 28: 10–21.

Gosner EL. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* 16: 183–190.

Green K, Osborne W. 2012. *A Field Guide to Wildlife of the Australian Snow Country*. New Holland (Australia) Pty Ltd, Sydney, Australia.

Hero JM, Gillespie G, Robertson P, Lemckert F. 2004. *Pseudophryne corroboree*. In: IUCN 2012. *IUCN Red List of Threatened Species*. Version 2012.2. Available: www.iucnredlist.org [Accessed: 08 March 2012].

Hunter DA. 2000. The conservation and demography of the Southern Corroboree Frog. Masters of Applied-Science Thesis, University of Canberra, Australia.

Hunter D, Pietsch R, Marantelli G. 2007. Recovery actions for the Southern and Northern Corroboree Frogs (*Pseudophryne corroboree* and *Pseudophryne pengilleyi*): Annual Report and Recommendations. Unpublished report to the Corroboree Frog Recovery team.

Hunter D, Osborne W, Marantelli G and Green K.1999. Implementation of a population augmentation project for remnant populations of the Southern Corroboree Frog (*Pseudophryne corroboree*). Pp. 158–167 In: *Declines and Disappearances of Australian Frogs*. Editor, Campbell A. Environment Australia, Canberra, Australia.

Hunter D, Osborne W, Smith M, McDougall K. 2009a. Breeding habitat use and the future management of the critically endangered Southern Corroboree Frog. *Ecological Management and Restoration* 10: S103–S109.

Hunter D, Pietsch R, Marantelli G, McFadden M, Harlow P. 2009b. Field Research, Recovery Actions and Recommendations for the Southern Corroboree Frog (*Pseudophryne corroboree*) Recovery Project. Report to the Murray Catchment Management Authority.

Hunter DA, Speare R, Marantelli G, Mendez D, Pietsch R,Osborne W. 2009c. Presence of the amphibian chytrid fungus *Batrachochytrium dendrobatidis* in the threatened corroboree frog populations in the Australian alps. *Diseases of Aquatic Organisms* 92: 209–216.

Hunter D, Marantelli G, McFadden M, Harlow P, Scheele B, Pietsch R. 2010. Assessment of re-introduction methods for the Southern Corroboree Frog in the Snowy Mountains region of Australia. Pp 72–76 In: *Global Re-Introduction Perspectives: additional case-studies from around the globe.*Editor, Soorae PS. IUCN/SSC Re-introduction Specialist Group, Abu Dhabi, United Arab Emirates.

Johnson ML, Berger L, Philips L, Speare R. 2003. Fungicidal effects of chemical disinfectants, UVlight, desiccation and heat on the amphibian chytrid, *Batrachochytrium dendrobatidis*. *Diseases of Aquatic Organisms* 57: 255–260.

Jorgensen CB. 1992. Growth and Reproduction. Pp. 439–466 In: *Environmental Physiology of the Amphibians*. Editors, Feder ME, Burggren WW. University of Chicago Press, Chicago, Illinois, USA.

Kaplan RH. 1987. Developmental plasticity and maternal effects of reproductive characteristics in the frog, *Bombina orientalis*. *Oecologia* 71: 273–279.

McCallum H. 2012. Disease and the dynamics of extinction. *Philosophical Transactions of the Royal Society B* 367: 2828–2839.

Mendelson JR. 2011. Shifted baselines, forensic taxonomy, and Rabbs' Fringe-limbed Treefrog: the changing role of biologists in an era of amphibian declines and extinctions. *Herpetological Review* 42(1): 21–25.

Moore JA. 1953. A new species of Pseudophryne from Victoria. *Proceedings of the Linnaean Society of NSW* 78: 179–180.

Osborne WS. 1989. Distribution, relative abundance and conservation status of Corroboree Frogs *Pseudophryne corroboree* Moore (Anura: Myobatrachidae). *Australian Wildlife Research* 6: 537–547.

Osborne WS, Hunter DA, Hollis GL. 1999. Population-declines and range contraction in Australian alpine frogs. Pp. 145–157 In: *Declines and Disappearances of Australian Frogs*. Editor, Campbell A. Environment Australia, Canberra, Australia.

Pengilley RK. 1973. Breeding biology of some species of *Pseudophryne* (Anura: Leptodactylidae) of the Southern Highlands, New South Wales. *Australian Zoologist* 18: 15–30.

Pengilley RK. 1992. Natural history of *Pseudophryne* spp. (Anura: Myobatrachidae) in the Southern High lands of N.S.W., Australia. *Sydney Basin Naturalist* 1: 9–29.

Pessier AP, Mendelson JR (Editors). 2010. *A Manual for Control of Infectious Diseases in Amphibian Survival Assurance Colonies and Reintroduction Programs.* IUCN/SSC Conservation Breeding Specialist Group: Apple Valley, Minnesota, USA.

Schloegel LM, Hero JM, Berger L, Speare R, McDonald K, Daszak P. 2006. The decline of the Sharp-snouted Day Frog (*Taudactylus acutirostris*):the first documented case of extinction by infection in a free-ranging wildlife species. *Ecohealth* 3:35–40.

Seymour RS, Geiser F, Bradford DF. 1991. Metabolic cost of development in terrestrial frog eggs (*Pseudophryne bibroni*). *Physiological Zoology* 64: 688–696.

Skerratt LF, Berger L, Speare R, Cashins S, McDonald-KR, Phillott AD, Hines HB, Kenyon N 2007. Spread of chytridiomycosis has caused the rapid global decline and extinction of frogs. *EcoHealth* 4:125–134.

Stuart SN, Chanson JS, Cox NA, Young BE,Rodrigues ASL, Fischman DL, Waller RW. 2004. Status and trends of amphibian declines and extinctions worldwide. *Science* 306: 1783–1796.

Sullivan BK, Ryan MJ, Verrell PA. 1995. Female choice and mating system structure. Pp. 469–517 In: *Amphibian Biology*. Volume 2. Social behaviour. Editors, Heatwole H, Sullivan BK. Surrey Beatty and Sons, Chipping Norton, New South Wales, Australia.

Thumm K, Mahony M. 2002. Evidence for continuous iteroparity in a temperate-zone frog, the red-crowned toadlet, *Pseudophryne australis* (Anura: Myobatrachi dae). *Australian Journal of Zoology* 50: 151–167.

Wells KD. 2007. *The Ecology and Behavior of Amphibians.* The University of Chicago Press. Chicago, Illinois, USA.

Woodhams DC, Bosch J, Briggs CJ, Cashins S, Davis LR, Lauer A, Muths E, Puschendorf R, Schmidt BR, Sheafor B, Voyles J. 2011. Mitigating amphibian disease: strategies to maintain wild populations and control chytridiomycosis. *Frontiers in Zoology* 8: 8.

Zippel K, Johnson K, Gagliardo R, Gibson R, McFadden M, Browne R, Martinez C, Townsend, E. 2011. The Amphibian Ark: A global community for *ex situ* conservation of amphibians. *Herpetological Conservation and Biology* 6: 340–352.

Received: 24 March 2013
Accepted: 19 June 2013
Published: 31 October 2013

Michael McFadden is the Supervisor of the Herpetofauna Department at Taronga Zoo, where he has worked for ten years since completing his Honours degree in Biology at the University of Technology, Sydney. He co-ordinates the Zoo's amphibian conservation programs and is also the Co-convenor of the ZAA Amphibian Taxon Advisory Group. His main interests are reptile and amphibian conservation, conservation breeding programs and reintroduction biology.

Raelene Hobbs has been working in the Herpetofauna Department at Melbourne Zoo since 2005. Her interest in amphibians began from a young age and over the years she has had many amazing opportunities to be involved with many different species of amphibians. Completing an Associate Diploma in Resource Management, volunteering and working with amphibians since 1998, Raelene is now the Amphibian Specialist at Melbourne Zoo. She is currently working with two critically endangered and one endangered Australian frog species, specializing in captive breeding, long-term husbandry, and population dynamics and breeding animals for release back into the wild.

Gerry Marantelli is the founder and owner of the Amphibian Research Centre, a private facility dedicated to the conservation and research of Australian threatened frogs. He has been heavily involved in amphibian conservation for over thirty years, including initiating the captive component of the corroboree frog program. Gerry also pioneered the use of shipping containers, or pods, for use in amphibian conservation programs.

Peter Harlow is the Manager of the Herpetofauna Division at Taronga Zoo, Sydney, and is currently involved in conservation programs for the Fijian Crested Iguana, two Critically Endangered Australian lizard species from Christmas Island and six Critically Endangered Australian frog species. He received his Ph.D. from Macquarie University on the ecology of temperature dependent sex-determination in Australian agamid lizards. Over his three decade-long career he has worked on ecology and conservation biology projects on a wide variety of reptile and amphibian species, mostly in Australia, but also working in Southern Africa, Indonesia, USA, Canada and Fiji.

Chris Banks has worked in zoos in Australia and the UK since 1969, with a primary focus on captive management and conservation of reptiles and amphibians. He currently manages Zoos Victoria's international conservation partnerships and provides strategic input to ZV's native threatened frog recovery programs.

David Hunter is a threatened species officer with the New South Wales Office of Environment and Heritage in Australia where his primary role is the management and implementation of threatened frog recovery programs. David has been involved in the corroboree frog recovery program since its conception in 1996.

CONTENTS

Administration, journal information (Instructions to Authors), and copyright notice. ………..…...… Inside front cover

Robert K. Browne, Katja Wolfram, Gerardo García, Mikhail F. Bagaturov, and Zjef J. J. M. Pereboom—Zoo-based amphibian research and conservation breeding programs. ………………………...……. 1

Marlen Wildenhues, Anna Rauhaus, Rike Bach, Detlef Karbe, Karin Van Der Straeten, Stefan T. Hertwig, and Thomas Ziegler—Husbandry, captive breeding, larval development and stages of the Malayan horned frog *Megophrys nasuta* (Schlegel, 1858) (Amphibia: Anura: Megophryidae). ………………………… 15

Anna Gawor, Anna Rauhaus, Detlef Karbe, Karin Van Der Straeten, Stefan Lötters, and Thomas Ziegler—Is there a chance for conservation breeding? *Ex situ* management, reproduction, and early life stages of the Harlequin toad *Atelopus flavescens* Duméril & Bibron, 1841 (Amphibia: Anura: Bufonidae). 29

Doris Preininger, Anton Weissenbacher, Thomas Wampula, and Walter Hödl—The conservation breeding of two foot-flagging frog species from Borneo, *Staurois parvus* and *Staurois guttatus*. …..……………………… 45

Devin Edmonds, Justin Claude Rakotoarisoa, Rainer Dolch, Jennifer Pramuk, Ron Gagliardo, Franco Andreone, Nirhy Rabibisoa, Falitiana Rabemananjara, Sahondra Rabesihanaka, and Eric Robsomanitrandrasana—Building capacity to implement conservation breeding programs for frogs in Madagascar: Results from year one of Mitsinjo's amphibian husbandry research and captive breeding facility. …... 57

Michael McFadden, Raelene Hobbs, Gerry Marantelli, Peter Harlow, Chris Banks, and David Hunter—Captive management and breeding of the Critically Endangered Southern Corroboree Frog (*Pseudophryne corroboree*) (Moore 1953) at Taronga and Melbourne Zoos. ……………………………………… 70

Table of Contents. ………………………………………………………………………………...... Back cover

VOLUME 5 2012 NUMBER 3